Biological Tissue Archive Studies

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BIOLOGICAL TISSUE ARCHIVE STUDIES

by

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ABSTRACT

Hyatt, W.H. J.D. Fitzsimons, M.J. Keir & D.M. Whittle. 1986. Biological tissue archive studies. Can. Tech. Rep. Fish. Aquat. Sci. 1497. pp. i-iv, 1-58.

The Great Lakes Fisheries Research Branch has continuously archived fish tissue homogenates from the Canadian Great Lakes since 1978. Fish samples collected and analyzed for the annual Contaminants Surveillance Program have also been archived for future retrospective analysis of recently identified contaminants. Additional samples have been archived for experiments designed to define preservation and long-term storage conditions required to maintain the integrity of chlorinated hydrocarbon residues in biological tissues.

Samples have been stored at -20°C, -40°C, -80°C, and -196°C. Preservation treatments including prefreezing, and a stepped rate of freezing, have been studied. Some samples were oven-dried at +60°C, prior to storing at room temperature.

Data indicate freezing at -80°C was preferential to storage at the other temperatures studied. Prefreezing and a stepped rate of freezing did not produce significantly better storage conditions for sample integrity.

Radiolabelled PCB was used to measure the efficiencies of three extraction methods as compared to a biological material oxidizer. The efficiencies of all extraction methods studied appeared to be low when compared to the radiolabelled PCB level recovered with a biological material oxidizer. Other preservation techniques studied included formalin and hydrochloric acid treatments of fish tissue. These techniques were found not to be adequate methods to maintain the integrity of the samples with respect to chlorinated hydrocarbon residues.

RESUMÉ

Hyatt, W.H. J.D. Fitzsimons, M.J. Keir & D.M. Whittle. 1986. Biological tissue archive studies. Can. Tech. Rep. Fish. Aquat. Sci. 1497. pp. i-iv, 1-58.

Depuis 1978, la Direction de la recherche sur les pêches dans les Grands Lacs a toujours conservé des homogénats de tissus de poissons provenant des Grands Lacs au Canada. Les échantillons de poissons recueillis et analysés dans le cadre du Programme annuel de surveillance de la pollution ont aussi été archivés à des fins d'analyse future rétrospective des contaminants identifiés dernièrement. D'autres échantillons ont été archivés en vue d'expériences visant à établir les conditions de conservation et d'entreposage à long terme nécessaires au maintien de l'intégrité des résidus d'hydrocarbures polychlorés dans les tissus biologiques.

Des échantillons ont été entreposés à -20°C, -40°C, -80°C et -196°C. On a étudié des traitements de conservation comprenant une pré-congélation et une congélation par étapes. Quelques échantillons ont été séchés à l'étuve à une température de +60°C avant d'être entreposés à la température ambiante.

D'aprés les données, il serait préférable de congeler les échantillons à -80°C plutôt que de les entreposer aux autres températures étudiées. La pré-congélation et la congélation par étapes n'offrent pas de conditions d'entreposage vraiment supérieures pour l'intégrité de l'échantillon.

Des BPC à marqueurs radioactifs ont été utilisés pour mesurer l'efficacité de trois méthodes d'extraction comparativement à un oxydant biologique. Toutes les méthodes d'extraction étudiées semblaient être peu efficaces lorsqu'on comparait leurs résultats à la teneur en BPC à marqueurs radioactifs récupérés à l'aide d'un oxydant biologique. Les traitements des tissus de poisson au formol et à l'acide hydrochlorique constituent d'autres méthodes de conservation. On a constaté que ces méthodes ne permettaient pas de maintenir l'intégrité des échantillons relativement aux résidus d'hydrocarbures polychlorés.

INTRODUCTION

Biological tissue archiving, the long term storage of tissues for retrospective analysis, is indispensable to an environmental monitoring program which is primarily concerned with substances that have been designated as hazardous or potentially hazardous. A tissue archive allows for retrospective monitoring of currently unidentified or recently identified toxic compounds, enabling contaminant trends to be defined over time and provides information of the trend in body burdens of these toxic compounds in the aquatic ecosystem. If retrospective monitoring is to reliably indicate the history of a contaminant, it requires that the integrity of the sample and that contaminant remain intact. Studies described in the following report were designed to define appropriate preservation and storage conditions required for maintaining for extended periods the integrity of chlorinated hydrocarbon residues, polychlorinated biphenyls (PCBs), and lipid in a variety of biological tissues. These storage and preservation methods should allow the greatest latitude to be adapted to future methods of analyses. The influence of factors such as field procedures, storage temperatures, and tissue types on specific contaminant residues must be determined and controlled in order to maintain representative tissue samples for inclusion in an archive. The term 'losses', as used in this report could actually mean: (i) real losses, (ii) lack of recovery, (iii) decreased extractability or, (iv) conversion to degradation products.

In this report, several independent studies have been conducted to address different aspects of a tissue archive. To facilitate ease of following the different 'phases', each will be dealt with independently.

PHASE I

A two-year study was initiated to examine the losses of chlorinated hydrocarbon residues, polychlorinated biphenyls (PCBs), and lipid in whole coho salmon (Oncorhynchus kisutch) homogenate samples stored at -20°C or -40°C. A 'prefreezing' step, using liquid nitrogen, was included to prevent the lipid portion of the sample from separating before freezing was complete.

PHASE II

To assess the archiving potential of invertebrates, bulk samples of a zooplankter, Mysis relicta, were stored at -40°C, -20°C or oven dried at +60°C and stored at room temperature. These samples were later analyzed for chlorinated hydrocarbon residues, PCBs, and lipid.

Phase III

This study was intended to investigate rapid losses of chlorinated hydrocarbon residues, PCBs and lipid from Mysis samples and to determine the effect of ultra-low storage temperatures (-80°C and -196°C) on sample integrity.

Usually, bulk Mysis samples were collected and transported to a laboratory or other work facility on day one, then held overnight in chilled lake water

until processed (sorted, packaged and frozen) the next day. Evidence from previous studies indicated some chlorinated hydrocarbon residue levels declined in Mysis samples from the time of sample collection to the time of sample processing. Previous experiments indicated that up to 40 percent of the body lipid could be lost in 24 hours, and 60 percent in 48 hours (Borgmann and Whittle, 1983).

PHASE IV

A further study was initiated to verify the rapid declines in chlorinated hydrocarbon residue levels observed in Mysis samples within 24 hours from the time of collection to the time of processing. Previous studies indicated some chlorinated hydrocarbon residue levels were higher in samples stored at -80°C compared to levels in samples stored at -196°C. This study was also intended to gather further information regarding these two storage conditions.

PHASE V

The purpose of this study was to determine if observed losses were real or if there was a reduction in the extractability of the chlorinated hydrocarbon residues analyzed. Radiolabelled PCB was used to determine the extraction efficiencies of three methods compared to the ^{14}C-PCB levels obtained when samples were analyzed with a Biological Material Oxidizer (BMO). Utilization of a radiotracer would allow the determination of the extraction efficiency of the three methods through liquid scintillation counting techniques. Prolonged storage of formalin preserved and acid-digested fish tissue samples was also studied.

MATERIALS AND METHODS

PHASE I

Five fall run coho salmon jacks, age 2+, were collected in the Credit River approximately five km upstream from Lake Ontario. These fish were stored whole at -25°C for five weeks before being processed. All fish were individually homogenized to provide a series of whole fish sample replicates. A consistent homogenate was obtained by passing each fish five times through a commercial meat grinder. Samples were taken from the bulk homogenate sample. The samples were stored in acetone/hexane-rinsed round, seamless tin containers. Half the samples of each of the series of homogenate replicates was immersed in liquid nitrogen (-196°C) for two minutes, after which all samples were stored at either -20°C or -40°C. This prefreezing step lowered the sample temperature to -20°C and was intended to prevent the lipid portion of the samples from separating before freezing was complete.

Pesticide analyses were performed by an independent contract laboratory at intervals of 1 month, 11 months, and 25 months after processing. A 2.5 gm sample was ground with 50 gm of sodium sulfate until it was free-flowing, followed by Soxhlet extraction for five hours with 25 percent ether in hexane.The extract was made up to 150 ml volume with hexane and 15 ml of this

was used for gravimetric lipid determination. The remaining extract was concentrated to about 5 ml on a roto-evaporator and cleaned up on two percent water-deactivated Florisil according to the method described by Reynolds and Cooper (1975).

A chromatographic column, containing 70 ml hexane, was packed with 30 g of Florisil and topped with 1 cm sodium sulfate. The hexane was drained to the top of the sodium sulfate and the sample extract was added. The first fraction was eluted with 180 ml hexane and contained DDE, PCB, chlorobenzenes, mirex, and photomirex. The second fraction was eluted with 300 ml of 30 percent methylene chloride in hexane and contained DDT, DDD, dieldrin, heptachlor epoxide, and chlordanes. Analyses were performed by electron capture gas-liquid chromatography. Capillary gas chromatography (GC) analysis was used for chlorobenzenes, mirex, and photomirex, and packed column GC for the remaining residues.

PHASE II

Mysis were collected with a modified epibenthic sled (Elster, 1933) at a site approximately 65 m deep off the Niagara River in Lake Ontario. The invertebrates were removed from the sediments by sieving the sample through either a 500 µm or a 1410 µm mesh screen. After rinsing, Mysis were placed in a 20-litre pail with 12 litres of water at 6°C, previously collected from 40 m. To maintain this temperature, plastic bags filled with ice had been added to the pail one hour before the addition of the Mysis. The water was aerated continuously until arrival on shore, where the pail was sealed for the one hour road trip to the laboratory.

Sample sets were processed and stored at intervals of approximately 24 hours, 45 hours, and 50 hours after collection. Each sample set consisted of one sample for each of the three storage conditions (-20°C, -40°C, and oven dried with subsequent storage at room temperature) and for each of the storage times (1, 20, and 35 months) for a total of nine replicate samples. Mysis were sorted, rinsed with distilled water and refrigerated at 3°C. The entrained water was extracted under vacuum in a Buchner funnel. When approximately 190 g of Mysis, enough for one set of samples, had been sorted and surface water removed, the bulk sample was placed in an iced container and homogenized at low speed for approximately 90 seconds with a Polytron tissue homogenizer. Six replicate samples were placed in individual, preweighed, solvent-rinsed glass jars with screw tops, weighed and placed in freezers. Three samples were stored at each of -20°C and -40°C. Three additional replicate samples were placed in solvent-rinsed aluminum weigh boats and stored in a drying oven at 60°C until all the moisture was removed. These samples were stored in solvent-rinsed borosilicate glass vials with screw caps. The vials were sealed with solvent-rinsed aluminum foil before being capped. The samples were stored at room temperature. Analyses for chlorinated hydrocarbon residues and PCBs were performed one month, 20 months, and 35 months after collection.

The samples were analyzed by the Water Quality Laboratory, Inland Waters Directorate, at the Canada Centre for Inland Waters, Burlington,

Ontario. Two different extraction methods were employed. Each method used 10 g of tissue. The first two sets of samples were extracted with acetonitrile and partitioned with petroleum ether. The organic extract was further cleaned up on a Florisil column on which the PCBs and pesticides were fractionated into four fractions. Each was then examined by gas liquid chromatography (GLC). The complete methodology is described in Analytical Methods Manual, Inland Waters Directorate, Water Quality Branch, Ottawa, Canada, 1979. For the final set of samples, sodium sulfate and methylene chloride were added to the tissue sample and the sample was extracted with a polytron tissue homogenizer. This slurry was decanted through an Allihn filter, containing anhydrous sodium sulfate, into a round bottom flask. The extract was reduced in volume then transferred to a graduated centrifuge tube (15 ml). The flask was rinsed with a mixture of cyclohexane and methylene chloride (1:1) and transferred to the centrifuge tube. This was repeated until the final volume in the centrifuge tube was 10 ml. The sample was centrifuged and a portion of the extract was processed for the analysis of the chlorinated hydrocarbons. The contaminants were separated from the lipid materials using an automated gel permeation chromatograph charged with Bio-beads SX-3, and an eluting solvent of a 1:1 methylene chloride - cyclohexane mixture. The lipid-free extract containing the contaminants was subsequently chromatographed on a silica gel column for the fractionation of the chlorinated hydrocarbons and PCB. This method is described in the National Water Quality Operations Manual, 1987.

PHASE III

Mysis samples for this study were collected from three sites on Lake Ontario with an epibenthic sled. At two sites, Niagara-on-the-Lake and Cobourg, some Mysis samples were placed in acetone/hexane-rinsed tin containers and then placed in liquid nitrogen (-196°C) within two hours of collection. The remaining bulk Mysis sample was stored overnight, submerged in 4°C lake water in darkened jars. The bulk sample was sorted and the Mysis placed in solvent-rinsed round, seamless tin containers 24 hours after collection. Some samples, from the Cobourg site, were stored at -80°C. The remaining Cobourg samples and all the Niagara-on-the-Lake samples were submerged in liquid nitrogen. Mysis from Port Credit were stored in liquid nitrogen either 24 or 28 hours after collection.

Pesticide analyses were performed by an independent contract laboratory. The chlorinated hydrocarbon residues were extracted from 5 g samples of Mysis tissue with methylene chloride and a polytron tissue homogenizer. The methylene chloride extract was reduced from 70 ml to about 5 ml on a rotary evaporator and then centrifuged at 2000 RPM for five minutes, with a portion of the extract taken for lipid analysis and the remainder processed for the analysis of the chlorinated hydrocarbon residues. The chlorinated hydrocarbon residues and the lipid were separated with an automated gel permeation chromatograph charged with Bio-beads SX-3, and an eluting solvent of a 1:1 methylene chloride - cyclohexane mixture.

The extract containing the contaminants was chromatographed on a silica gel column for the fractionation of the chlorinated hydrocarbons and PCBs.

The silica gel fractions were subsequently analyzed by capillary column gas chromatography with electron capture detection for the chlorinated hydrocarbons and by packed column gas chromatography for PCB, mirex, and photomirex. This methodology was developed by the Water Quality Laboratory, Inland Waters Directorate, Canada Centre for Inland Waters, Burlington, Ontario and is described in the National Water Quality Operations Manual, 1987.

PHASE IV

Mysis samples were collected from an 80 m depth site in Lake Ontario, 10 km north-west of Niagara-on-the-Lake. When the bulk samples were brought on board, they were placed on a 1050 μm mesh nitex screen and kept on ice until processed. Some samples were sorted and frozen within two hours of collection. The remaining bulk samples were left overnight, on nitex screens, suspended above ice. These samples were placed in a 4°C cooler, in the dark. The following day, the bulk samples were processed and stored under the same conditions used on collection day. Samples were frozen on collection day (T_0) and processing day (T_0 + 1) at -196°C, -80°C, and -80°C for 24 hours before subsequent transfer to -196°C for storage, and at -196°C for 24 hours before transfer to -80° for storage (Fig. 1). The purpose of the latter two storage conditions was to investigate the effect of a stepped freezing rate (i.e., -80°C to -196°C) and prefreezing (i.e., -196°C to -80°C).

Samples frozen at -196°C were placed in liquid nitrogen, while those frozen at -80°C were stored on dry ice until arrival at the laboratory where they were placed in an ultra-low freezer, operating at -80°C. Samples were frozen for 34 to 41 days before pesticide analyses were initiated.

Samples for storage at -196°C were placed in solvent rinsed round seamless tin containers with solvent rinsed aluminum foil between the container and the lid. Samples for -80°C storage were placed in solvent rinsed flint glass jars with solvent rinsed aluminum foil between the jar and lid.

A contract laboratory performed the analyses for chlorinated hydrocarbon residues and PCBs. The procedures were similar to those described in Phase III. Moisture content was also determined on unfrozen Mysis samples for collection day and processing day.

PHASE V

Twelve laboratory-reared rainbow trout (Salmo gairdneri), each weighing approximately 1500 g, were subdivided into two equal groups. One group received a high dose of ^{14}C-PCB (1.0 mg.kg^{-1}), while the other group received a low dose (0.1 mg.kg^{-1}). The radiolabelled PCB was Aroclor 1254 with a specific activity of 31.1 mCi.nmole^{-1}. It was uniformly labelled, with an isomer balance the same as commercial 1254. Aroclor 1254 was chosen because studies carried out on Great Lakes fish suggest that the PCB composition is similar to commercially prepared Aroclor 1254 (Veith, 1975). An 80 percent assimilation rate was used to develop dosage rates. The PCB, combined with capelin oil in gelatin capsules, was administered to MS-222

anesthetized fish by passage through a modified intubation tube into the stomach.

After a two-week equilibration period the fish were sacrificed. Fillet samples were removed from high-dose fish. The skin and surface fat were removed. The fillets were homogenized in a commercial meat grinder, subsampled, and stored for up to 146 weeks at -20°C. The high-dose whole fish samples were re-combined with the remaining fillet homogenates and subsampled as whole fish samples. The six low-dose fish were also homogenized and subsampled for the various treatments. Whole fish homogenate samples were stored for up to 146 weeks at -20°C, -40°C, and -196°C for the extraction methods studied. The effect of formalin preservation on levels of radiolabelled PCB was also studied. Formalin (five percent w/w) was added to the remaining high-dose homogenate after all non-preserved samples were removed. To correct preserved samples for the additional weight of the formaldehyde, sample weights were multiplied by a factor of 0.95. These preserved samples were stored at -20°C for up to 146 weeks.

Acid dissolution was another method studied for long-term storage of biological tissue. Some high-dose whole fish homogenate samples were dissolved in hydrochloric acid and stored at room temperature for up to 147 weeks. The acid was intended to break down the tissue matrix and stop any microbial activity. When the acid-stored samples were extracted, the procedure was the same as that for the HCl extraction method discussed later.

All whole fish and fillet samples for -20°C and -40°C storage were placed in round seamless tin containers, then frozen in liquid nitrogen and stored at the appropriate temperature. Samples for liquid nitrogen storage (-196°C) were sealed in borosilicate glass ampules. There were five replicates of 25 g for each treatment.

Biological Material Oxidizer

The Biological Material Oxidizer (BMO) was used for preparing biological samples containing carbon-14 tracer for liquid scintillation counting. The BMO combusts any biological and most organic materials in a stream of oxygen. The combustion side of the BMO furnace is maintained at 900°C and initiates the oxidation of organic materials and converts the sample to the gaseous state. The bulk of the material is combusted to carbon dioxide and water, but partially combusted molecular fragments are also present. In addition, gaseous forms of the halogens, sulphur, phosphorous, and nitrogen might occur. Metals, salts, and materials of very high melting points remain in the combustion tube boat (Kravetz, M.A. 1971).

The gaseous combustion products are swept through the catalyst bed, which is maintained at 700°C, by the oxygen gas stream. The catalyst bed converts to carbon dioxide and water those compound fragments which have not been completely oxidized. The gaseous forms of the halogens, sulphur, and phosphorous are removed by the catalyst bed; only oxygen, nitrogen, and nitrogen oxides are expelled from the combustion tube exit.

Carbon-14 dioxide is trapped in a carbon dioxide trapping agent. In trapping carbon-14 dioxide, the gas reacts with an organic amine to form a solid which is soluble in, and compatible with, liquid scintillation counting solutions.

The BMO is capable of a 98 percent or greater recovery of carbon-14 based upon 50 mg mannitol with specific activity of 100 disintegrations per minute (dpm) or greater per mg of added standard. A correction factor may be calculated based on dpm added and dpm recovered. The corrected total dpm is the value used in all subsequent calculations. The calibration procedure corrects for the differences in operator technique, changes in gas flow rate, changes in catalyst bed conditions, differences in counting solutions, and differences in temperature. The corrected total dpm obtained using the BMO were used as reference levels to evaluate the efficiencies of the three extraction methods described in the following sections. The samples were counted in a liquid scintillation counter (Beckmann, Model LS8100) with the sample channels ratio quench correction. The measure of activity was in disintegrations per minute. This was converted to disintegrations per minute per gram ($dpm.g^{-1}$) of tissue used. This unit of measurement was used to compare the amount of ^{14}C-PCB recovered from the fish tissue after variations in extraction methodology, storage time, and storage temperature.

Extraction Methods

(i) Hydrochloric Acid/Methylene Chloride (HCl/MC) Extraction

In the first extraction method, 100 ml of hydrochloric acid was added to 10 gm of fish homogenate in a 125 ml wide-mouth glass jar. After shaking the sample overnight, the HCl slurry was transferred to a 250 ml separatory funnel. This solution was extracted into another 250 ml separatory funnel with three 25 ml methylene chloride rinses. The methylene chloride was washed with 50 ml of XAD water (Coburn et al., 1977). After the methylene chloride and the XAD water were separated, the methylene chloride was passed through 5 cm of anhydrous sodium sulfate, in an Allihn filter, into a 500 ml round bottom flask. Five ml of 2,2,4-trimethylpentane was added to the flask, the volume reduced and adjusted, and the activity counted according to the method followed in the second extraction.

(ii) Acetonitrile Extraction

This extraction method consisted of an acetonitrile extraction in a blender, with a partitioning step into petroleum ether. Ten g of fish homogenate were transferred to a commercial blender. Acetonitrile (120 ml) was added and the solution was blended at medium-high speed for 15 minutes. After the solid phase settled, the acetonitrile extract was poured into an Allihn filter containing prewashed celite covering the sintered glass filter. To the residue in the blender, another 120 ml of acetonitrile and 40 ml of distilled water were added and blended for ten minutes. This solution was filtered through the Allihn filter. Another 60 ml of acetonitrile was added to the homogenate and blended for ten

minutes. All the residue was transferred, with acetonitrile rinses, to the Allihn filter. Suction was applied to remove the solvent from the residue in the filter. The acetronitrile extract was transferred with petroleum ether rinses into a one liter separatory funnel and diluted with distilled water to adjust the aqueous content to 20 percent. The resulting mixture was extracted first with 150 ml and then twice with 75 ml of petroleum ether. The combined petroleum ether extract was washed with 200 ml of distilled water. The water was discarded and the extract was passed, under suction, through an anhydrous sodium sulfate (10 to 15 g) column using a 500 ml round-bottom flask as a receiver. The content of the flask was evaporated to 2 or 3 ml in a rotary evaporator (Buchi), then transferred to a 15 ml graduated centrifuge tube using petroleum ether rinses. The centrifuge tube was previously calibrated. The volume was adjusted to 10 ml, with 1 ml being transferred to a borosilicate glass scintillation vial containing 10 ml of Phase Combining System (PCS). The activity was measured in a scintillation counter.

(iii) Acetone/Hexane Extraction

The third method involved homogenizing 5 g of fish tissue, 5 g of anhydrous sodium sulfate and 10 ml each of acetone and hexane in a 50 ml culture tube. The sample was blended at high speed using a Polytron tissue homogenizer. The liquid portion of the sample was decanted into an Allihn filter, containing 5 cm of sodium sulfate, connected to a 500 ml round bottom flask. After weak suction was applied to the Allihn filter, the solid portion of the sample was rinsed into the filter with two 10 ml hexane rinses. Suction was increased for this final extraction. Approximately 5 ml of 2,2,4-trimethylpentane (iso-octane) was added to the round-bottom flask and the volume was reduced to 5 ml on a rotary evaporator. This solution was transferred to a 15 ml graduated, calibrated, centrifuge tube, using 2,2,4-trimethylpentane rinses. The volume was adjusted to 10 ml. One ml of the extract was transferred, after vortex action mixing, to a borosilicate glass vial containing 10 ml PCS and activity was counted in a scintillation counter.

Background Activity

Laboratory reared rainbow trout were taken from the same tanks as the experimental fish and were used to define the background levels of radioactivity. These control fish were subjected to the same analytical procedures as the dosed fish. The amount of radioactivity found in these control fish was incorporated as a correction factor in the calculations to define the amount of ^{14}C-PCB recovered from the experimental fish. These background ^{14}C levels are listed in Table 1.

RESULTS

PHASE I

The prefreezing step did not have a significant effect on the levels of chlorinated hydrocarbon residues (Table 2). Therefore, all samples within a storage period/temperature framework were treated as replicates.

There were no significant differences in lipid levels (Fig. 2) after storage for one year at -20°C or for two years at -40°C, compared to the initial levels. Significant lipid losses ($P<0.05$) of 20.9 percent (0 to 39 percent) occurred after two years of storage at -20°C. There was a gradual reduction in all lipid levels over this two year storage period. The data are summarized in Table 3.

There was a gradual decrease in PCB levels (Fig. 3) over the two year storage period at -20°C. The loss was not significant after one year of storage. After the samples had been stored for two years the change was significant with a mean loss of 21.4 percent (0 to 42.8 percent). There were no significant PCB losses after two years of storage at -40°C.

There were significant losses of both mirex and photomirex (Fig. 4) regardless of storage temperature. Individual sample losses of mirex ranged from 30.2 to 38.9 percent and 43.7 to 53.8 percent after one and two years of storage respectively. There were no significant losses of photomirex after one year of storage, but losses of 37 percent occurred during the second year.

Significant p,p' DDD losses (Fig. 5) of 14 percent (0 to 30 percent) and 56 percent (42 to 65 percent) were observed after storage at -20°C for one and two years respectively compared to the initial levels. There were no significant losses after one year of storage at -40°C. Significant p,p'DDD losses of 47.7 percent (31 to 54 percent) occurred after two years of storage at -40°C.

Changes in the concentration of γ chlordane, oxychlordane, and heptachlor epoxide, after prolonged storage, were not calculated because initial levels were near the detection limit of the analytical method. Levels of p,p' DDE, p,p' DDT, α chlordane, hexachlorobenzene, and dieldrin fluctuated randomly during the two year study.

PHASE II

The chlorinated hydrocarbon residue data statistically analyzed in this study were p,p'DDE, p,p'DDT, dieldrin, mirex, hexachlorobenzene (HCB), and PCB. After one month of storage, HCB levels in the oven-dried samples were significantly lower ($P<0.01$) than in samples frozen at -40°C. HCB concentrations in these samples were 31.9 percent (22.6 to 38.1 percent) lower than in samples stored at -40°C. No other chlorinated hydrocarbon residue level was significantly lower, regardless of storage condition (-40°C, -20°C, or +60°C with subsequent storage at room temperature).

After 20 months of storage there were no statistically significant differences in any of the contaminant levels compared to initial levels. However, there was a considerable interval between extraction and cleanup for these samples, resulting in some variable data.

After 35 months of storage all the chlorinated hydrocarbon residues analyzed were below the limit of detection of .004 $\mu g.g^{-1}$ and .09 $\mu g.g^{-1}$ for chlorinated hydrocarbons and PCBs, respectively.

PHASE III

Samples from Niagara-on-the-Lake, stored at -196°C on either collection day (T_0) or processing day (T_0 + 1), were compared. Collection day samples and processing day samples were stored for 141 days and 140 days, respectively before analyses were made. The results are presented in Table 4. There were significant losses ($P<0.01$) in T_0 + 1 samples compared to T_0 samples of PCB (65.6 percent), p,p'DDE (69.2 percent), dieldrin (50 percent), lipid (51.3 percent) (Fig. 6), o,p'DDT (78.9 percent), oxychlordane (55.6 percent), mirex (61.1 percent) and heptachlor epoxide (55 percent) (Fig. 7). There were no significant differences in levels of p,p'DDT, p,p'DDD, γ chlordane, α chlordane, photomirex, or hexachlorobenzene. All bulk samples left overnight in water had an oily film on the water surface suggesting a loss of lipid.

Mysis from Port Credit were stored for 150 days before analyses. There were significant ($P<0.05$) p,p'DDD losses of 57.9 percent (21 to 74 percent) for samples stored 28 hours after collection as compared to those stored 24 hours after collection. There were no other significant chlorinated hydrocarbon residue losses observed for these two groups (Table 5).

There were losses of chlorinated hydrocarbon residues in Cobourg Mysis samples stored at -196°C on processing day (T_0 + 1) when compared to samples stored on collection day (T_0) (Fig. 8 and 9). All samples were stored for 114 to 120 days. There were significant losses ($P<0.05$) of p,p'DDE (34.8 percent, 31 to 40 percent), lipid (48.9 percent, 41 to 55 percent), o,p'DDT (38.6 percent, 29 to 57 percent), α chlordane (25.9 percent, 7 to 44 percent), oxychlordane (22.6 percent, 3 to 35 percent) and heptachlor epoxide (22.6 percent, 19 to 35 percent). Non-significant losses ($P<0.1$) of PCB, dieldrin and photomirex also occurred. There were no significant losses for p,p'DDD, p,p'DDT, γ chlordane, mirex, and hexachlorobenzene.

Samples stored at -80°C on T_0 + 1 were compared to those stored at -196°C on T_0 (Fig. 10). There were no significant losses identified for either sampling group. However, there were non-significant losses ($P<0.1$) of oxychlordane, photomirex, and heptachlor epoxide.

A further comparison was made between samples stored on processing day at either -196°C or -80°C (Fig. 11). The -196°C samples had lower levels of mirex, hexachlorobenzene, and p,p'DDE, and significantly ($P<0.05$) lower levels of PCB and lipid. Levels of PCB and lipid were lower by 26.2 percent (19 to 35 percent) and 43.4 percent (35 to 50 percent) respectively.

Levels of PCB, p,p'DDE, and lipid were compared for the three storage conditions (Fig. 12). In each case, the samples stored at -196°C on processing day had lower levels than samples stored at -196°C on collection day or at -80°C on processing day.

The mean levels obtained for the chlorinated hydrocarbon residues and lipid for the three storage conditions are presented in Table 6.

PHASE IV

Levels of most chlorinated hydrocarbon residues determined for samples stored immediately upon collection (T_0) were greater than levels found in samples processed and stored one day later (T_0+1) (Table 7A,7B). In 37.5 percent of these cases, losses were significant at $P<0.05$ (Table 8). Lipid levels were also lower for samples stored on the subsequent processing day. The levels of αchlordane reported for these series of samples were at the analytical detection limit for any storage condition in this phase.

Chlorinated hydrocarbon residue levels were compared for samples stored on the second day. There were no significant differences in these residue levels regardless of sample storage conditions. Losses of these residues had already occurred when the Mysis were held overnight. There were significant differences ($P<0.05$) in residue levels in samples stored at different temperatures on collection day. Chlorinated hydrocarbon residues experiencing these differences were HCB, heptachlor epoxide, dieldrin, p,p'DDE, o,p'DDT, p,p'DDT, γ chlordane, oxychlordane, and mirex (Table 9). See Table 7A for actual levels.

In previous studies some chlorinated hydrocarbon residue levels were found to be lower in samples stored at -196°C compared to samples stored at -80°C. In this study significantly ($P<0.05$) lower levels of HCB, heptachlor epoxide, p,p'DDT, and dieldrin were found in samples stored on collection day at -196°C compared to samples stored at -80°C (Fig. 13). Only mirex and p,p'DDE showed significantly higher levels when stored at -196°C compared to storage at -80°C, on collection day.

A graduated freezing rate (-80°C to -196°C) and flash prefreezing (-196°C to -80°C) did not significantly reduce chlorinated hydrocarbon residue losses in comparison to levels determined for samples frozen at either -196°C or -80°C.

The moisture content of Mysis was determined for both collection day replicates and for samples held overnight above ice. The mean sample moisture content for collection day of 70.43 percent (±3.32 S.D.) and for the following processing day of 73.35 percent (±1.63 S.D.) were not significantly different.

PHASE V

Low Dose

Biological Material Oxidizer

The low dose samples were analyzed, using a BMO, after 11, 95 and 146 weeks of storage (Fig. 14). There were no significant differences in measured ^{14}C-PCB levels compared to initial levels, except for the samples stored at -40°C for 95 weeks. These samples had ^{14}C-PCB levels significantly higher than the initial levels. All samples analyzed at 95 weeks of storage had ^{14}C-PCB levels significantly higher ($P<0.05$) than levels in samples stored for either 11 weeks or 146 weeks under similar storage temperatures.

Hydrochloric Acid/Methylene Chloride (HCl/MC) Extraction

Samples were stored for 95 and 146 weeks before being extracted with hydrochloric acid and methylene chloride. All samples stored for 95 weeks had ^{14}C-PCB levels significantly lower ($P<0.01$) than the initial levels (Fig. 15). The ^{14}C-PCB levels were lower by 35, 43, and 30 percent for storage at -20°C, -40°C, and -196°C respectively. All samples stored at -196°C were accidentally destroyed after 95 weeks when the glass ampules cracked in the liquid nitrogen container. After 146 weeks of storage, the samples stored at -40°C had ^{14}C-PCB levels similar to the initial levels, even though samples stored at -40°C for 95 weeks showed significantly lower levels. Samples stored at -20°C for 146 weeks had ^{14}C-PCB levels significantly lower ($P<0.05$) than the initial levels. These levels were 27.5 percent lower than the initial levels. The levels observed after 95 and 146 weeks of storage were not significantly different from each other.

All samples stored for 95 weeks before being extracted with HCl/Methylene Chloride had ^{14}C-PCB levels significantly lower ($P<0.01$) than corresponding samples combusted in the BMO (Fig. 18). These levels were lower by 40.0, 50.4, and 35.4 percent when samples were stored at -20°C, -40°C, and -196°C respectively.

Initial ^{14}C-PCB levels and those after 146 weeks of storage were not significantly different when combusted in the BMO or extracted with HCl/MC.

Acetonitrile Extraction

Samples were extracted, using acetonitrile, after 95 weeks and 146 weeks of storage. There were no significant differences in levels of ^{14}C-PCB in any of the samples analyzed, regardless of storage period or temperature (Fig. 16). However, the initial levels and the levels in samples stored for 95 weeks were significantly lower ($P<0.05$) than levels in corresponding BMO combusted samples (Fig. 18).

The initial ^{14}C-PCB levels were significantly ($P<0.05$) lower than the initial BMO levels by 27.6 percent. The acetonitrile extracted ^{14}C-PCB levels were significantly lower than the corresponding BMO combusted sample

levels by 32.3, 37.1, and 32.6 percent when stored for 95 weeks at -20°C, -40°C, and -196°C respectively.

Acetone/Hexane Extraction

Samples were extracted with acetone and hexane after 95 and 146 weeks of storage. Levels of ^{14}C-PCB in samples stored at -20°C and -40°C were significantly higher ($P<0.01$) than the initial levels (Fig. 17). Levels in samples stored at -196°C were not significantly different from initial levels. The initial radiolabelled PCB levels obtained for samples extracted with acetone and hexane were significantly lower ($P<0.05$) than levels obtained when samples were combusted in the BMO (Fig. 18). Levels measured in the acetone/hexane extracts were 27.7 percent lower than levels obtained using the BMO.

Levels of ^{14}C-PCB in samples stored for 95 and 146 weeks at -20°C, then extracted with acetone and hexane were significantly higher ($P<0.05$) than levels in corresponding samples combusted in the BMO.

Samples held for 146 weeks at -40°C before being extracted with acetone and hexane had significantly ($P<0.05$) higher levels of ^{14}C-PCB than did corresponding BMO combusted samples.

High Dose

Fillet

After 147 weeks of storage at -20°C there was a significant ($P<0.01$) ^{14}C-PCB loss of 21.4 percent in fillet samples compared to the initial levels measured. Initially, ^{14}C-PCB levels in fillet samples represented 45.7 percent of the whole fish concentration.

Preservation with Formalin

Analyses of the formalin preserved tissues were made after 6, 11, 93, and 146 weeks of storage at -20°C. There was no significant loss of ^{14}C-PCB after up to 93 weeks of storage (Fig. 19), but a significant loss of 22.6 percent ($P<0.01$) occured after 146 weeks of storage, compared to the initial levels.

High dose samples that were stored at -20°C were compared to samples that were preserved with formalin before being stored at the same temperature (Fig. 20). There were no significant differences in levels of ^{14}C-PCB recovered when samples were stored up to 93 weeks. After 146 weeks of storage there were significant ($P<0.01$) losses of 20.6 percent in formalin preserved samples stored at -20°C versus samples simply stored frozen at -20°C.

Biological Material Oxidizer

High dose, whole fish samples were stored for 6, 11, 95, and 146 weeks before being combusted in the BMO. None of the ^{14}C-PCB levels for any storage

period were significantly different from each other. An exception was in samples stored for 6 weeks at -196°C (Fig. 21) where levels were significantly higher ($P<0.05$) than all other levels obtained.

HCl/Methylene Chloride (HCl/MC) Extraction

Samples were extracted with HCl/MC after being stored for 95 or 146 weeks. Samples stored at -20°C had significantly lower ($P<0.01$) ^{14}C-PCB levels than the initial concentration (Fig. 22). Levels were 21.4 and 17.2 percent lower after storage for 95 and 146 weeks respectively. The mean concentration in samples stored at -40°C for 95 weeks decreased by 24 percent compared to the initial levels measured. However, levels in samples stored at -40°C for 146 weeks were not significantly different from the initial levels. Radiolabelled PCB levels in samples stored at -196°C were not significantly different from the initial levels.

The initial high dose level of ^{14}C-PCB recovered using the HCl/MC extraction was 14.7 percent less than that recovered using the BMO (Fig. 27). Subsequent recoveries after 95 weeks of storage utilizing an HCl/MC extraction were 33.0, 32.4, and 11.1 percent less than that recovered with the BMO technique when samples were stored at -20°C, -40°C, and -196°C respectively. After 146 weeks of storage, the HCl/MC-extracted ^{14}C-PCB levels were 27.5 and 16.0 percent lower than the corresponding BMO samples stored at -20°C and -40°C respectively. Levels in samples stored for 146 weeks at -196°C were not significantly different.

Hydrochloric Acid Dissolution and Storage

The acid/tissue solution was thick and black. The inside of the storage jars, in contact with the solution, was covered in a black film which was difficult to remove. This suggests that some ^{14}C-PCB may have also adhered to the glass, making it unavailable for extraction.

Samples dissolved in HCl had significantly lower ($P<0.01$) initial ^{14}C-PCB levels (-14.7 percent) than did the samples combusted in the BMO (Fig. 23). After 95 weeks of storage, extracted samples had ^{14}C-PCB levels 30.3 percent lower than the initial HCl stored sample levels and 41.1 percent lower than the corresponding BMO-combusted sample levels. After 146 weeks of storage there was a significant loss ($P<0.01$) of 73.6 percent of the ^{14}C-PCB compared to the initial HCl extraction levels obtained. There was a loss of 76.9 percent when compared to corresponding samples combusted in the BMO. The ^{14}C-PCB values obtained after storage for 146 weeks may have been lower than the true values. A clearly defined separation between the HCl and the methylene chloride was difficult to obtain during the extraction procedure. This poor separation may have prevented the collection of all the ^{14}C-PCB available.

After 95 weeks of storage at room temperature, HCl dissolved samples had ^{14}C-PCB levels significantly lower ($P<0.01$) than the initial levels (30.3 percent) or the levels found in samples stored at -196°C (31 percent) before acid dissolution (Fig. 24).

After 146 weeks of storage, the levels in acid stored samples were significantly less ($P<0.01$) than all other levels. These levels were 73.6 percent lower than the initial levels and 62.2 percent lower than the levels found in acid dissolved samples after 95 weeks of storage.

Acetone/Hexane Extraction

There was no significant difference between initial levels of ^{14}C-PCB compared to levels in samples after 95 and 146 weeks of storage. The single exception was significantly higher ^{14}C-PCB levels found in samples that had been stored for 146 weeks at -196°C (Fig. 25).

The ^{14}C-PCB levels obtained when the fish tissue samples were extracted with acetone/hexane were significantly lower than levels in corresponding samples combusted in the BMO, again with exception of the samples stored for 146 weeks at -196°C (Fig. 27). The ^{14}C-PCB levels in these acetone/hexane extracted samples were not significantly different from the levels found in corresponding samples combusted in the BMO. These levels were however significantly lower than the initial levels found in the BMO-combusted samples. Initial acetone/hexane-extracted levels were 28.8 percent lower than the initial BMO levels. After 95 weeks of storage, the acetone/hexane extracted ^{14}C-PCB levels were 24.6, 22.5, and 25.4 percent lower than the corresponding BMO-combusted samples when stored at -20°C, -40°C, and -196°C respectively. Samples stored at -20°C and -40°C for 146 weeks and then extracted with acetone/hexane had ^{14}C-PCB levels 16.3 and 10.0 percent lower than the corresponding BMO-extracted samples.

Acetonitrile Extraction

Samples were extracted with acetonitrile after 95 weeks and 146 weeks of storage. The ^{14}C-PCB levels obtained were not significantly different from the initial levels except in samples stored at -20°C for 146 weeks (Fig. 26). The initial levels were 15.2 percent lower than the levels in samples stored for 146 weeks at -20°C.

All ^{14}C-PCB levels obtained with acetonitrile extraction were significantly ($P<0.05$) lower than levels found in corresponding BMO-combusted samples (Fig. 27). The initial levels were 30.9 percent lower. After 95 weeks of storage, samples extracted with acetonitrile had levels that were 32.5, 19.5, and 23.5 percent lower when stored at -20°C, -40°C, and -196°C respectively. In comparison, after 146 weeks, sample levels were 16.5, 12.9, and 17.3 percent lower when storage was at -20°C, -40°C, and -196°C respectively.

Overall Comparison of Extraction Methods for High Dose Samples

Extraction methods were compared initially, after 95, and 146 weeks of storage (Fig. 27). All high dose samples stored at -20°C, -40°C, and those stored at -196°C for 95 weeks had significantly lower ($P<0.05$) ^{14}C-PCB levels than the corresponding BMO levels, regardless of extraction method. Samples stored at -196°C and extracted after 146 weeks with HCl/methylene chloride or acetone/hexane were not significantly different from the corresponding BMO

level, but the levels were significantly lower than the initial BMO levels by 15.4 percent and 13.6 percent, respectively.

DISCUSSION AND CONCLUSIONS

Phase I

The fluctuating levels of some chlorinated hydrocarbon residues may be due to changes in extractability with storage time and temperature, changes in extraction efficiency, contamination of samples or glassware, degradation products, or non-homogenous samples. The residue levels among replicates for a particular time-temperature condition were relatively consistent, which would indicate a factor or factors affecting the samples as a whole.

Results indicate that storage at -40°C is adequate to maintain some chlorinated hydrocarbon residue levels. However, in general, storage temperatures of -20°C and -40°C are not adequate to maintain the integrity of many chlorinated hydrocarbon residues in coho salmon whole fish homogenate samples over a prolonged storage period (i.e. >100 weeks). Prefreezing in liquid nitrogen did not have a significant effect on ensuring sample integrity.

Phase II

The collection and preparation of samples for this study contributed to variations in the data. Although this study left some unresolved questions some directions for future studies became apparent. Based on observations during field collections, it is recommended that Mysis not be held in lake water after collection. These fragile creatures die quickly, followed by rapid deterioration. This physical deterioration is evidenced by a surface 'oil' slick on the storage water. This loss of lipid probably contributed to reduced levels of some chlorinated hydrocarbon residues. After sorting, the Mysis were rinsed with distilled water and adhering water was extracted under vacuum. This would contribute to further lipophilic residue losses.

The time between sample collection and sample processing must be kept to a minimum. If not, some lipid and associated chlorinated hydrocarbon residues are probably lost even before the sample is processed.

Control of as many variables as possible must be maintained in order to ascertain the effects of time and temperature on the integrity of chlorinated hydrocarbon residues in biological material.

Phase III

Many chlorinated hydrocarbon residue levels decreased from the time the Mysis were collected to the time the samples were processed, i.e. overnight. This confirms the observation made in Phase II and reinforces the recommendation of immediate processing of Mysis samples in the field to prevent loss of lipophilic compounds as a result of rapid sample deterioration. When samples were stored at -196°C on collection day and on processing day, there was a

loss of some chlorinated hydrocarbon residue levels in samples stored on processing day. When additional samples were stored at -80°C on processing day, the results were contradictory to what was expected. Storage at a temperature less than -80°C would be assumed to be at least as beneficial as storage at -80°C. Therefore, some changes to sample quality may have occurred at this extremely low temperature.

Some chlorinated hydrocarbon residues may have been lost because of violent reactions to immersion in liquid nitrogen or some may have been lost due to holding samples overnight before processing them. The rates of freezing and thawing at these low temperatures, as well as container material, should be more thoroughly investigated. Our studies involved sample immersion in liquid nitrogen. Other investigators (Stoeppler et al. 1984, Wise et al. 1984) have employed liquid nitrogen vapour as the storage medium. Studies comparing storage in liquid nitrogen and liquid nitrogen atmosphere should be investigated. Also, the practice of storing bulk Mysis samples overnight in chilled lake water should be studied to ascertain the extent of overnight contaminant losses when this manner of preliminary storage is employed.

Phase IV

The results showed decreases in levels of chlorinated hydrocarbon residues and lipid in samples that were left overnight before they were processed and frozen. See Phases II and III for recommendations on collection and storage of Mysids.

There were no significant differences in chlorinated hydrocarbon residue levels among the several storage conditions utilized on processing day. This was probably due to the fact that losses of these residues had already occurred during the period of overnight storage.

Based on the results of this short-term study, immediate freezing on collection day at -80°C seems to be the best method for obtaining the real time chlorinated hydrocarbon residue levels in Mysis. Sample freezing in the field can be facilitated by using dry ice. A 22 kg block of dry ice will last for several days in a properly insulated container. Freezing at -80°C rather than -196°C is recommended in consideration of the residue levels obtained, the ease of sample storage in the field after collection and the cost and convenience of long-term storage at -80°C.

Phase V

The ^{14}C-PCB discussed in this study is presumed to have remained as ^{14}C-PCB and not changed to another ^{14}C-labelled compound.

The combustion of radiolabelled samples in a BMO is an uncomplicated technique. Some problems may be encountered that result in low ^{14}C recoveries. Conditions generating low ^{14}C recovery could be the use of a standard which is volatile at room temperature, incomplete combustion of the sample, fluctuations in oxygen flow rate, and improper packing of the combustion tube. Some problems may produce higher DPM than were originally

put in. This may occur due to chemiluminescence of the trapping agent, contamination of the sample, and fluorescence. However, if reasonable care is taken, these problems can be kept to a minimum.

More variability may be encountered in the three extraction procedures employed, compared to the BMO method. This is especially evident in the low dose samples, where slight alterations in such things as vacuum pressure or extraction end points may significantly alter ^{14}C-PCB levels obtained. Procedural variability between analysts would also have some influence on the amount of ^{14}C-PCB recovered. The variability in the low ^{14}C-PCB dose results may indicate a need for higher doses in extraction studies such as this. The high dose samples are able to withstand more variability between analysts and procedures without significant consequences.

For the high dose samples, the extraction methods generally recovered significantly less of the radiolabelled PCB than did the BMO.

Preservation with formalin was not considered to be a good method of long term storage for fish tissue. Radiolabelled PCB levels remained stable for 95 weeks, but after this significantly less ^{14}C-PCB was recovered.

Prolonged storage of acid-dissolved fish tissue is not recommended because of the low recovery rates of ^{14}C-PCB.

This study dealt with the storage of radiolabelled PCB and the results may not necessarily be applicable to other compounds.

GENERAL RECOMMENDATIONS

Some general conclusions can be drawn from these studies concerning the collection, handling, and processing of samples for archiving and retrospective chlorinated hydrocarbon analysis.

(i) **Fish**

Upon capture, fish should be isolated immediately from all sources of external contamination. Fish can be held temporarily in clean plastic tubs and then wrapped in solvent-rinsed aluminum foil as soon as possible. Fish should be chilled and frozen, whole, as soon as possible after collection and kept frozen until processed. In the field dry ice cut into sections about five centimeters thick can be layered between the fish. This method will rapidly freeze most of the fish.

Frozen fish should be cut into manageable pieces on a commercial meat saw equipped with a stainless steel blade and then homogenized thoroughly using a commercial meat grinder. The samples should be processed and refrozen as soon as possible. Stainless steel or Teflon coated equipment and utensils should be used for all phases of sample preparation, where possible.

Samples should be held in solvent-rinsed glass jars. The storage vessels are almost filled, then covered with solvent-rinsed aluminum foil before being capped. Shrink seals are used to prevent any loosening of the cap due to handling or possible expansion and contraction. This may occur as freezer temperatures oscillate, with the resulting change in gas volume in the storage vessel purging the head space of volatile compounds.

In the fish tissues studied, storage temperatures of -40°C or warmer were not adequate to maintain the integrity of chlorinated hydrocarbon residues, for prolonged storage periods.

(ii) **Invertebrates (*Mysis relicta*)**

Upon collection, remove *Mysis* from lake water and hold them in screens, above ice, in the dark. Process the samples as soon as possible after collection. If it is not possible to process all the samples soon after collection, it may be useful to process a subsample immediately. A comparison can be made later to determine the effects of storage on residue levels. Use stainless steel utensils for sorting, picking, etc. Store samples in solvent-rinsed tin or aluminum containers covered with solvent-rinsed aluminum foil before being tightly capped and apply shrink seals to the containers. Keep all samples frozen until immediately prior to analysis.

When the samples are ready to be analyzed, they should be thoroughly stirred to ensure homogeneity before the subsamples are taken.

Storage at -80°C seems to be the preferred storage temperature for *Mysis*.

(iii) **Laboratories**

Schedules for analysis should be confirmed with the contract laboratory. Sample storage temperature must be maintained when transportating samples from storage area to analyses area. Logistics problems may be encountered if the contract analytical laboratory does not have the storage temperature capabilities required. In this case, samples may only be transported to the laboratory immediately prior to extraction and analysis.

During long term storage studies, methodology changes should be avoided. If changes do occur, the effect on residue levels reported must be confirmed so the levels of chlorinated hydrocarbons can be compared for the different methodologies.

The contract laboratory selected should be required to report all quality assurance data generated during the period of the contract. In addition blind quality assurance samples should be included in the submissions to ensure a satisfactory level of performance. The laboratory should clearly identify the precise schedule of sample extraction and analysis.

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TABLE 1: Background ^{14}C activity found in control rainbow trout.

Extraction Method	Tissue Type	N	dpm.g^{-1}
BMO	Whole fish	5	239
Acetone/Hexane	Whole fish	2	126
Hydrochloric Acid	Whole fish	2	6
Acetonitrile	Whole fish	2	126
BMO	Fillet	5	536

TABLE 2: Mean chlorinated hydrocarbon residue levels in coho salmon whole fish homogenate samples that were frozen immediately at -20°C or -40°C, or underwent a prefreezing (-196°C) step first.

	INITIAL				ONE YEAR				TWO YEARS			
	-20°C		-40°C		-20°C		-40°C		-20°C		-40°C	
	Storage	Prefrozen	Storage	Prefrozen	Storage	Prefrozen	Storage	Prefrozen	Storage	Prefrozen	Storage	Prefrozen
	$ug.g^{-1}$	$ug.g^{-1}$	$ug.g^{-1}$	$ug.g^{-1}$	$ug.g^{-1}$	$ug.g^{-1}$	$ug.g^{-1}$	$ug.g^{-1}$	$ug.g^{-1}$	$ug.g^{-1}$	$ug.g^{-1}$	$ug.g^{-1}$
PCB	2.613 ±.536	2.421 +.499	2.521 ±.551	2.519 ±.492	2.351 ±.471	2.243 ±.375	2.581 ±.443	2.502 ±.457	2.043 ±.395	1.912 ±.359	2.352 ±.450	2.334 ±.458
p,p'DDD	.089 ±.015	.083 ±.016	.090 ±.015	.084 ±.014	0.076 ±.011	.072 ±.009	.079 ±.010	.083 ±.017	.040 ±.008	.036 ±.005	.046 ±.005	.045 ±.007
Photomirex	.044 ±.016	.040 ±.012	.038 ±.012	.040 ±.014	.042 ±.006	.039 ±.006	.041 ±.007	.041 ±.007	.025 ±.005	.022 ±.004	.028 ±.006	.027 ±.008
Mirex	.117 ±.032	.105 ±.034	.111 ±.037	.114 ±.036	.072 ±.017	.071 ±.013	.078 ±.014	.078 ±.015	.054 ±.014	.051 ±.007	.063 ±.013	.058 ±.022

N = 10

TABLE 3: Mean levels of chlorinated hydrocarbon residues and lipid in coho salmon homogenate samples over a two year storage period.

Storage Temperature	Storage Time	PCB ppm	p,p'DDD ppm	Mirex ppm	Photomirex ppm	Lipid %
-20°C	Initial	2.516±.514	0.086±.015	0.111±.033	0.042±.014	7.63 ±1.35
	One Year	2.297±.418	0.074±.010	0.071±.015	0.041±.006	7.008±1.092
	Two Years	1.977±.374	0.038±.007	0.053±.010	0.024±.005	6.033±1.167
-40°C	Initial	2.52± .508	0.087±.015	0.113±.037	0.039±.013	7.945±1.320
	One Year	2.541±.440	0.081±.014	0.078±.014	0.041±.007	7.82 ±1.000
	Two Years	2.343±.442	0.046±.006	0.060±.018	0.029±.006	6.985±1.310

N = 20

TABLE 4: Mean levels of chlorinated hydrocarbon residues and lipid in *Mysis* from Niagara-on-the-Lake, stored at -196°C.

	(T_0) Collection Day $ug.g^{-1} \pm$ S.D.	(T_0+1) Processing Day $ug.g^{-1} \pm$ S.D.
Hexachlorobenzene	.004±.001	.003±.001
Heptachlor Epoxide	.004±.001	.002±.001
Dieldrin	.017±.002	.009±.003
p,p'DDE	.063±.007	.020±.002
o,p'DDT	.004±.001	.001±.001
p,p'DDD	.006±.000	.003±.003
p,p'DDT	.012±.004	.008±.003
α chlordane	.003±.001	.002±.001
γ chlordane	.003±.002	.002±.001
Oxychlordane	.005±.001	.002±.000
Mirex	.009±.001	.004±.001
Photomirex	.002±.001	.001±.000
PCB	.240±.020	.083±.026
% Lipid	8.58 ±1.839	4.18 ±1.323

N = 4

TABLE 5: Mean levels of chlorinated hydrocarbon residues and lipid in Mysis from Port Credit, stored at -196°C.

	Time #1* $ug.g^{-1}$ ± S.D.	Time #2** $ug.g^{-1}$ ± S.D.
Hexachlorobenzene	.002±.001	.001±.000
Heptachlor Epoxide	.003±.001	.002±.001
Dieldrin	.010±.005	.010±.004
p,p'DDE	.026±.010	.030±.008
o,p'DDT	.002±.001	.002±.001
p,p'DDD	.004±.001	.002±.001
p,p'DDT	.005±.002	.005±.002
α chlordane	.001±.001	.002±.001
γ chlordane	.001±.001	.001±.001
Oxychlordane	.003±.001	.003±.001
Mirex	.003±.001	.004±.001
Photomirex	.001±.000	.001±.001
PCB	.083±.026	.114±.030
% Lipid	5.38 ±0.781	5.79 ±1.393

* 24 hours after collection

** 28 hours after collection

N = 4

TABLE 6: Mean levels of chlorinated hydrocarbon residues and lipid in Mysis from Cobourg.

	Collection Day	Processing Day	
	-196°C	-196°C	-80°C
	$ug.g^{-1}$ ± S.D.*	$ug.g^{-1}$ ± S.D.**	$ug.g^{-1}$ ± S.D.*
Hexachlorobenzene	.004±.001	.003±.000	.004±.001
Heptachlor Epoxide	.006±.001	.005±.001	.005±.001
Dieldrin	.030±.007	.022±.005	.027±.005
p,p'DDE	.093±.016	.061±.004	.081±.017
o,p'DDT	.007±.002	.004±.001	.005±.001
p,p'DDD	.008±.002	.006±.001	.007±.002
p,p'DDT	.011±.004	.012±.005	.011±.004
α chlordane	.005±.001	.004±.001	.005±.001
γ chlordane	.002±.000	.003±.001	.002±.000
Oxychlordane	.006±.001	.005±.001	.005±.001
Mirex	.010±.002	.008±.001	.010±.002
Photomirex	.003±.001	.002±.001	.002±.001
PCB	.286±.092	.183±.017	.248±.036
% Lipid	11.40 ±3.003	5.83 ±0.754	10.30 ±3.358

* N = 5

** N = 4

TABLE 7A: Mean levels of chlorinated hydrocarbon residues in *Mysis* from Niagara-on-the-Lake ($ug.g^{-1}$ ± S.D.).

	Collection Day (T_0) Storage Conditions			
	-196°C	-80°C	-80°C to -196°C*	-196°C to -80°C**
Hexachlorobenzene	.000	.004±.000	.004±.001	.004±.000
Heptachlor Epoxide	.009±.001	.013±.000	.007±.001	.012±.001
Dieldrin	.031±.001	.036±.002	.024±.003	.033±.004
p,p'DDE	.131±.014	.085±.011	.109±.012	.088±.011
o,p'DDT	.005±.001	.005±.001	.003±.001	.006±.001
p,p'DDD	.008±.000	.007±.001	.007±.001	.006±.003
p,p'DDT	.009±.003	.012±.000	.010±.001	.012±.002
α chlordane	.001±.000	.001±.000	.001±.000	.001±.000
γ chlordane	.007±.001	.008±.001	.006±.001	.007±.002
Oxychlordane	.009±.001	.011±.002	.007±.001	.011±.001
Mirex	.018±.002	.013±.004	.016±.001	.014±.002
Photomirex	.007±.002	.005±.002	.006±.000	.005±.001
PCB	.379±.034	.372±.041	.344±.059	.390±.042
% Lipid	8.35 ±.364	8.28 ±1.169	8.20 ±1.306	9.18 ±.847

N = 5

* -80°C to -196°C Storage at -80°C for 24 hours before transfer to -196°C for remaining storage period.

** -196°C to -80°C Storage at -196°C for 24 hours before transfer to -80°C for remaining storage period.

TABLE 7B: Mean levels of chlorinated hydrocarbon residues in *Mysis* from Niagara-on-the-Lake ($ug.g^{-1}$ ± S.D.).

	Processing Day (T_0+1) Storage Conditions			
	-196°C	-80°C	-80°C to -196°C*	-196°C to -80°C**
Hexachlorobenzene	.001±.002	.003±.002	.003±.002	.003±.001
Heptachlor Epoxide	.006±.002	.009±.003	.007±.002	.009±.002
Dieldrin	.022±.007	.028±.008	.021±.005	.027±.004
p,p'DDE	.098±.034	.073±.011	.088±.025	.065±.009
o,p'DDT	.004±.001	.003±.000	.003±.001	.004±.001
p,p'DDD	.005±.002	.004±.000	.004±.002	.005±.002
p,p'DDT	.008±.003	.006±.001	.008±.003	.009±.002
α chlordane	.001±.000	.001±.000	.001±.000	.001±.000
γ chlordane	.005±.002	.006±.002	.005±.002	.004±.001
Oxychlordane	.006±.002	.009±.002	.007±.002	.009±.001
Mirex	.015±.005	.013±.004	.014±.004	.013±.003
Photomirex	.006±.002	.005±.001	.005±.001	.004±.001
PCB	.291±.106	.302±.069	.284±.093	.284±.032
% Lipid	6.39 ±2.710	7.28 ±1.553	6.04 ±1.733	6.32 ±0.818

N = 5

* -80°C to -196°C Storage at -80°C for 24 hours before transfer to -196°C for remaining storage period.

** -196°C to -80°C Storage at -196°C for 24 hours before transfer to -80°C for remaining storage period.

TABLE 8: Chlorinated hydrocarbon residues exhibiting significantly higher levels on collection day compared to levels on processing day, for comparable storage conditions.

	-196°C	-80°C	-80°C to -196°C*	-196°C to -80°C**
Heptachlor Epoxide	**	**		***
Dieldrin	**	*		**
p,p'DDE	*			***
o,p'DDT	**	***		***
p,p'DDD	**	***	*	
p,p'DDT		***		**
γ chlordane	**			***
Oxychlordane	**			**
PCB		*		***
% lipid			*	***
N = 5			P < 0.01	***
			P < 0.05	**
			P < 0.1	*

* -80°C to -196°C Storage at -80°C for 24 hours before transfer to -196°C for remaining storage period.

** -196°C to -80°C Storage at -196°C for 24 hours before transfer to -80°C for remaining storage period.

TABLE 9: Storage temperatures that produced significantly different levels of chlorinated hydrocarbon residues in samples stored on collection day.

	-196°C	-80°C	-80°C to -196°C*	-196°C to -80°C**
Hexachlorobenzene				
Heptachlor Epoxide				
Dieldrin				
p,p'DDE				
o,p'DDT				
p,p'DDT				
γ chlordane				
Oxychlordane				
Mirex				

* -80°C to -196°C Storage at -80°C for 24 hours before transfer to -196°C for remaining storage period.

** -196°C to -80°C Storage at -196°C for 24 hours before transfer to -80°C for remaining storage period.

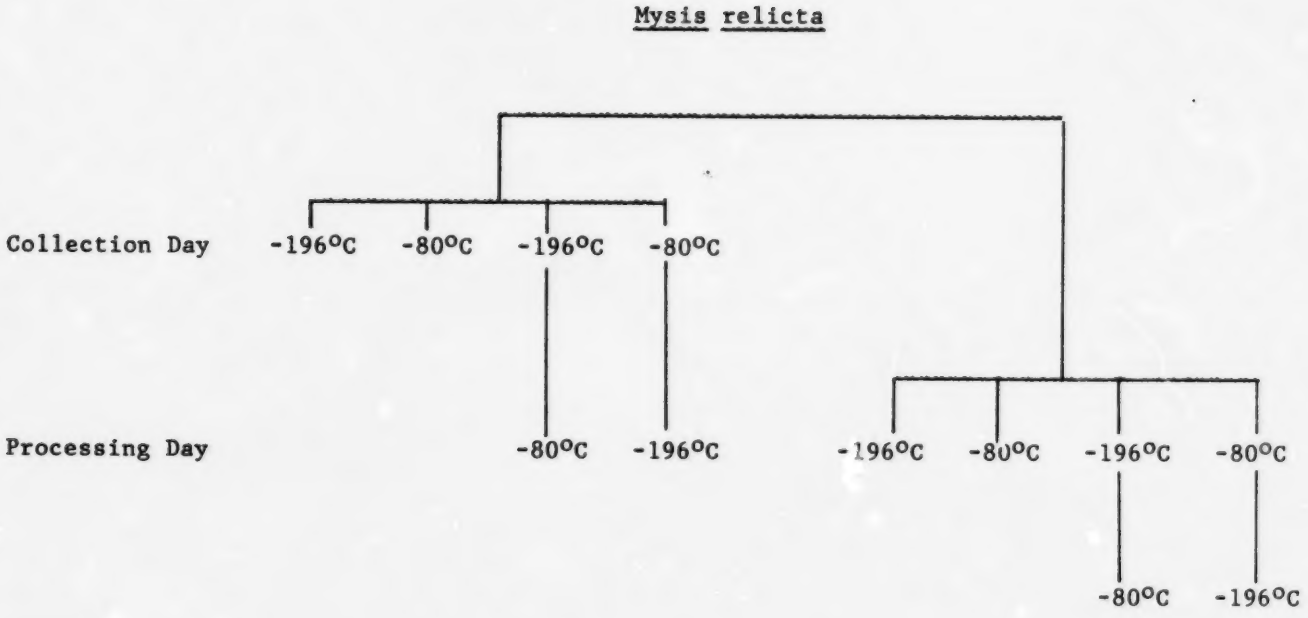

Figure 1. Schematic diagram of study outline indicating storage temperatures and storage initiation times.

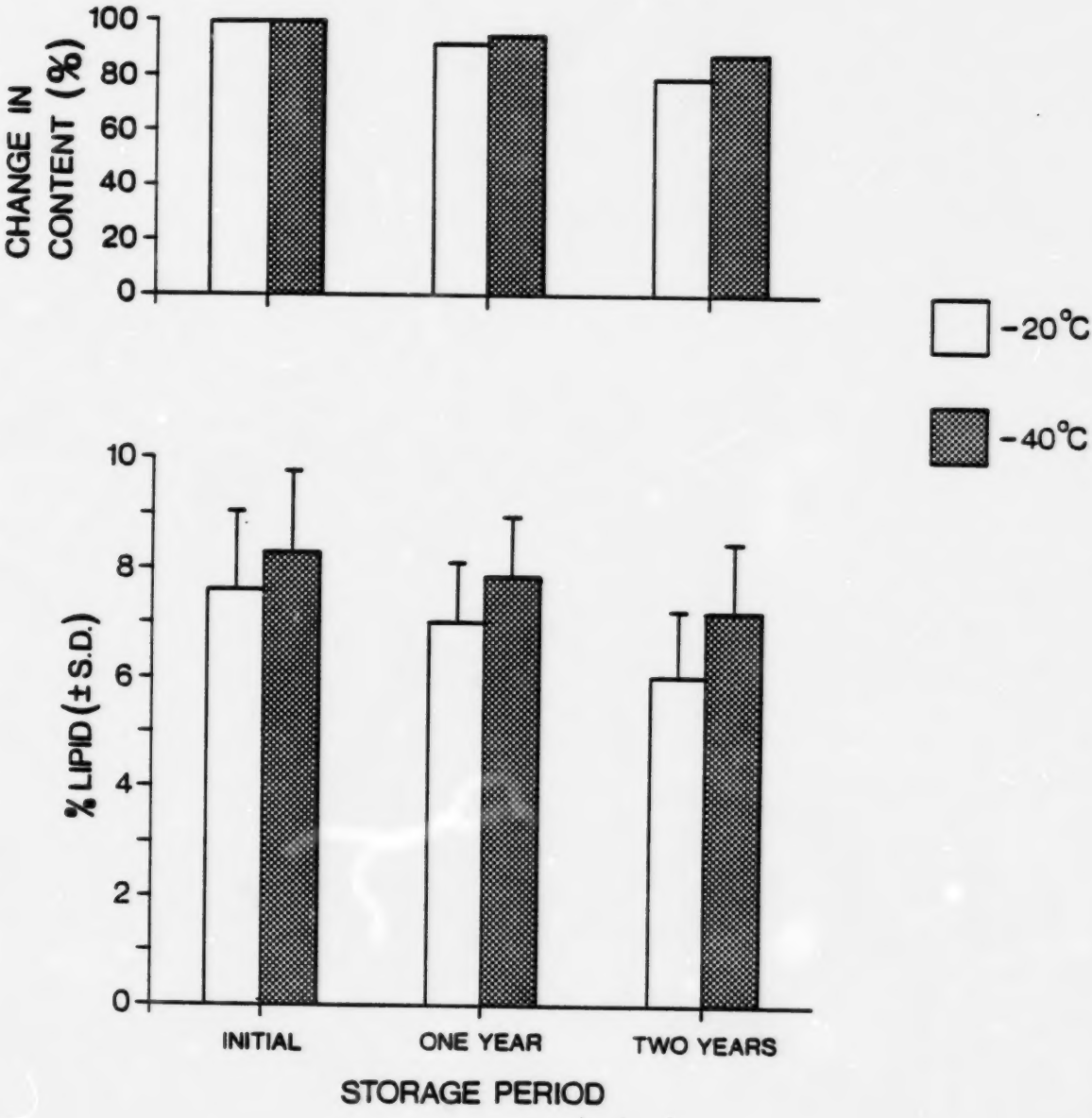

Figure 2. Levels of lipid in coho salmon homogenates over a two-year storage period.

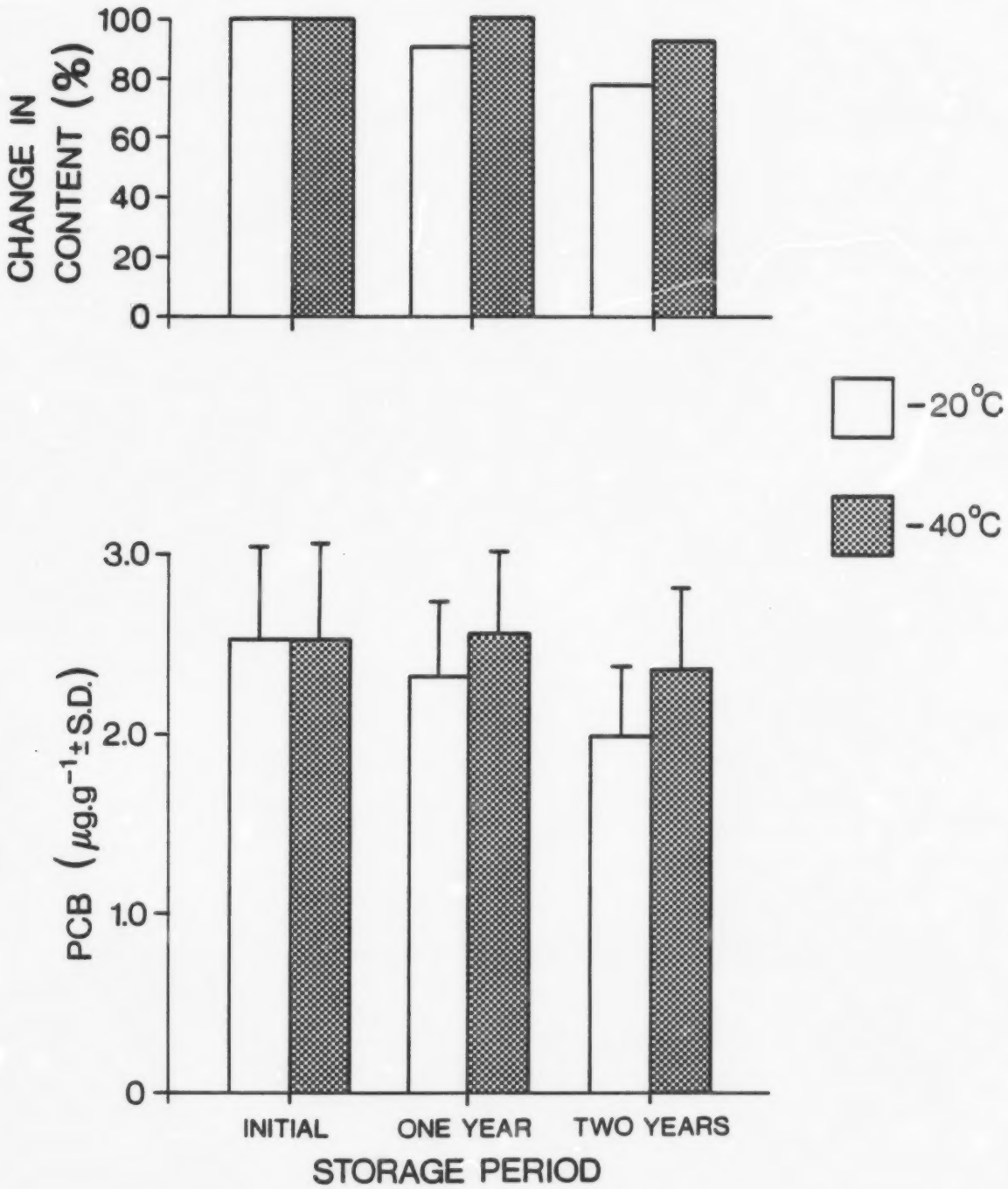

Figure 3. Levels of PCB in coho salmon homogenates over a two-year storage period.

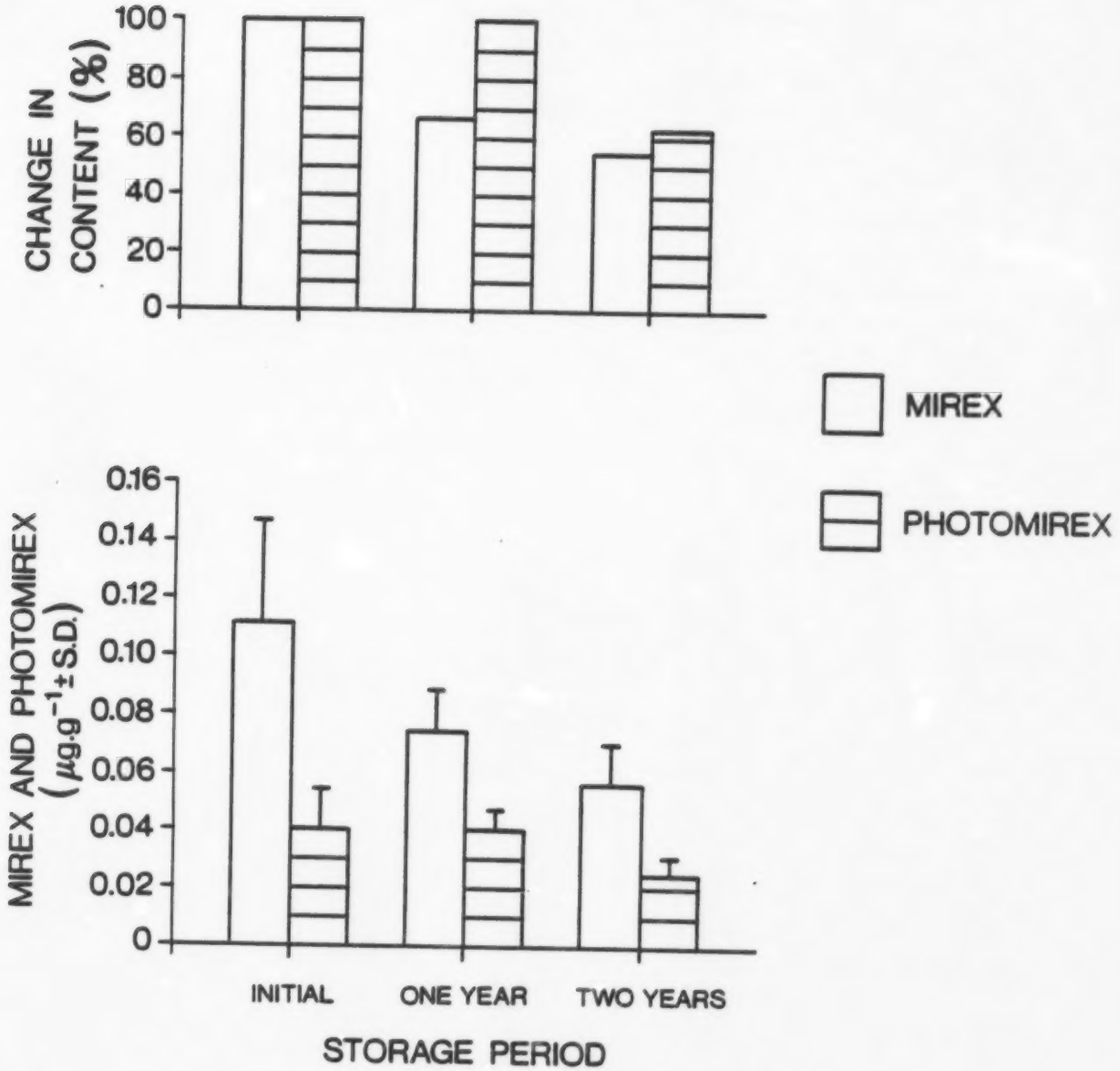

Figure 4. Levels of mirex and photomirex in coho salmon homogenates over a two-year storage period.

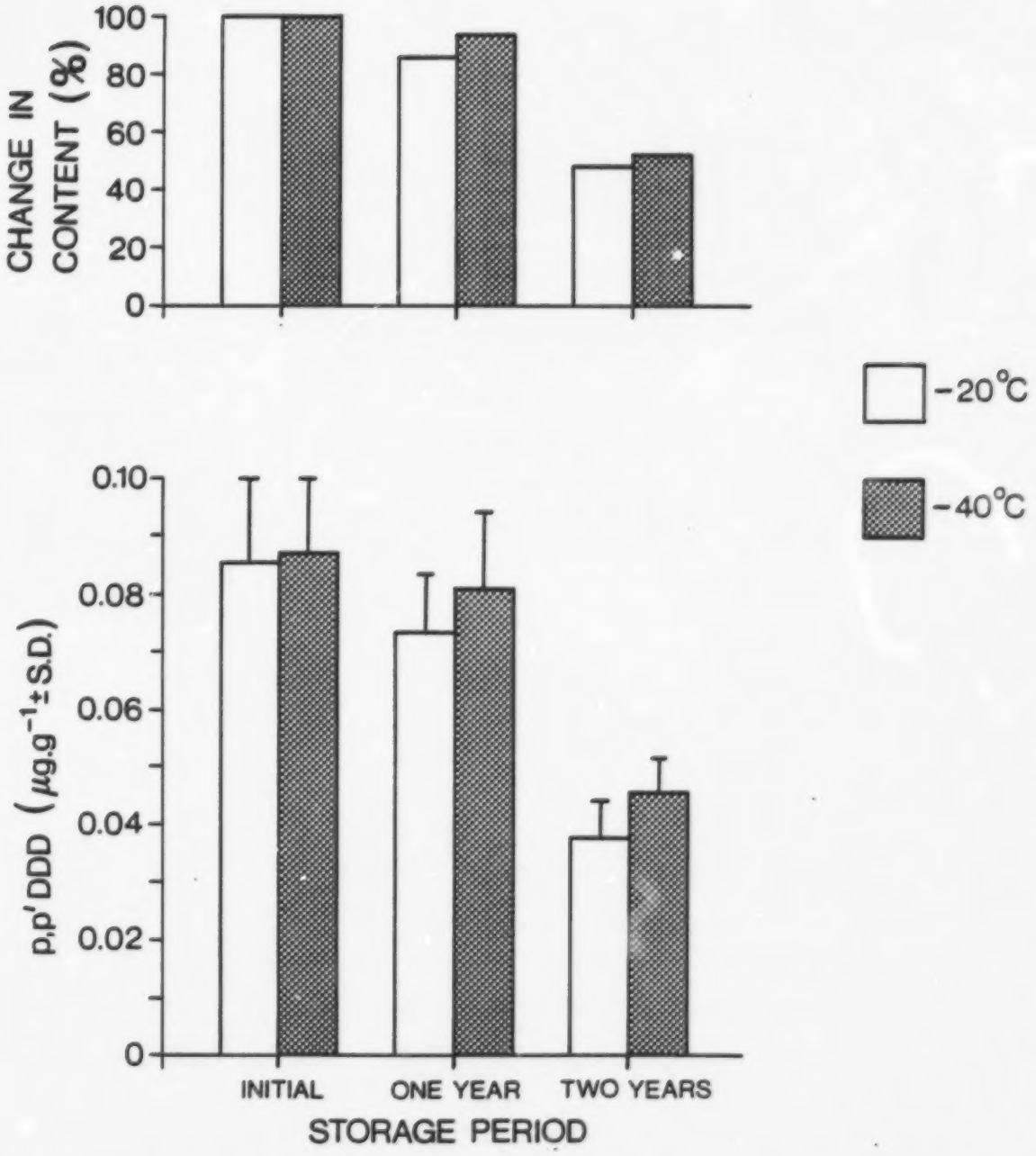

Figure 5. Levels of p,p'DDD in coho salmon homogenates over a two-year storage period.

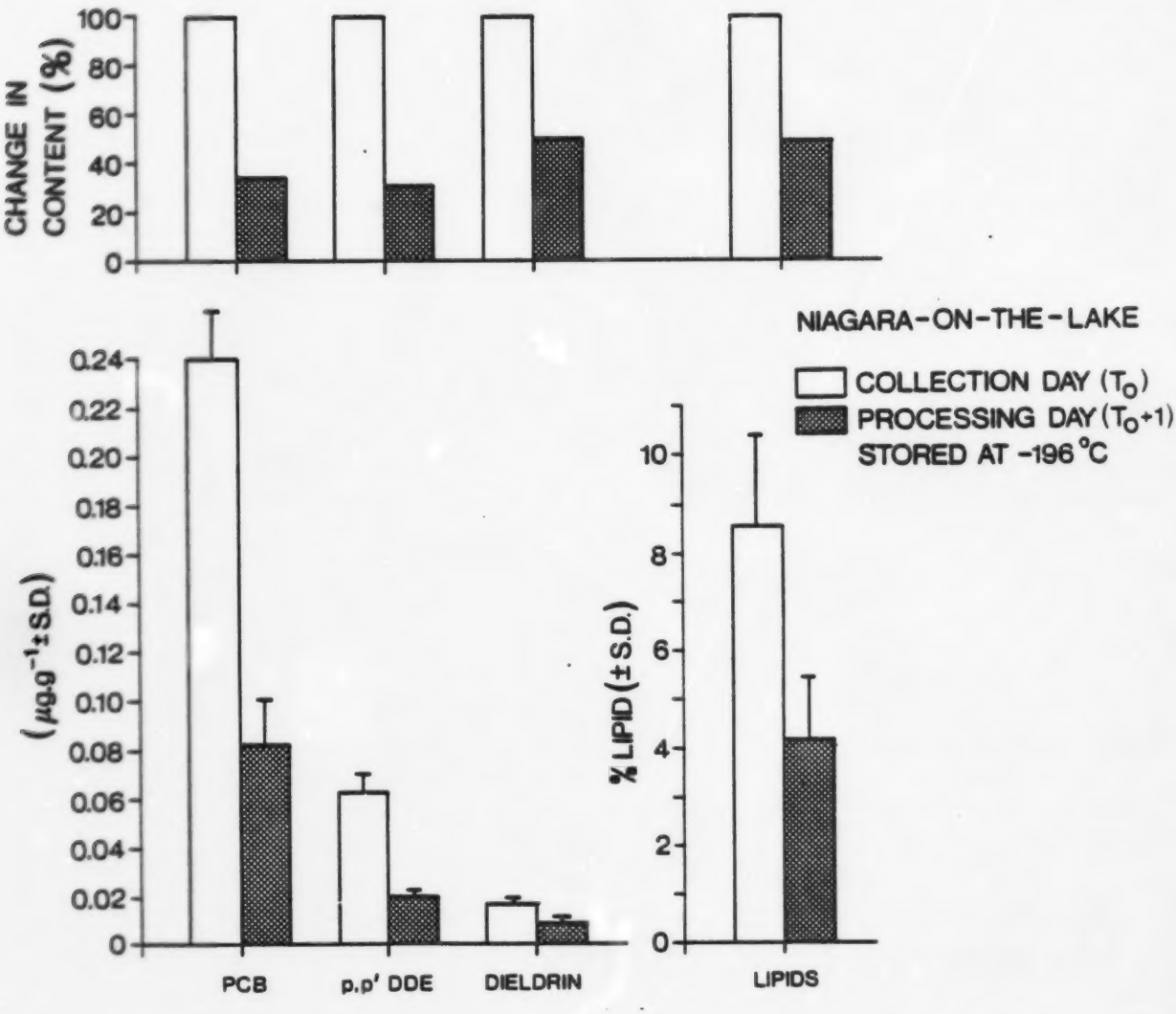

Figure 6. Levels of chlorinated hydrocarbons, PCB and lipid in Mysis from Niagara-on-the-Lake.

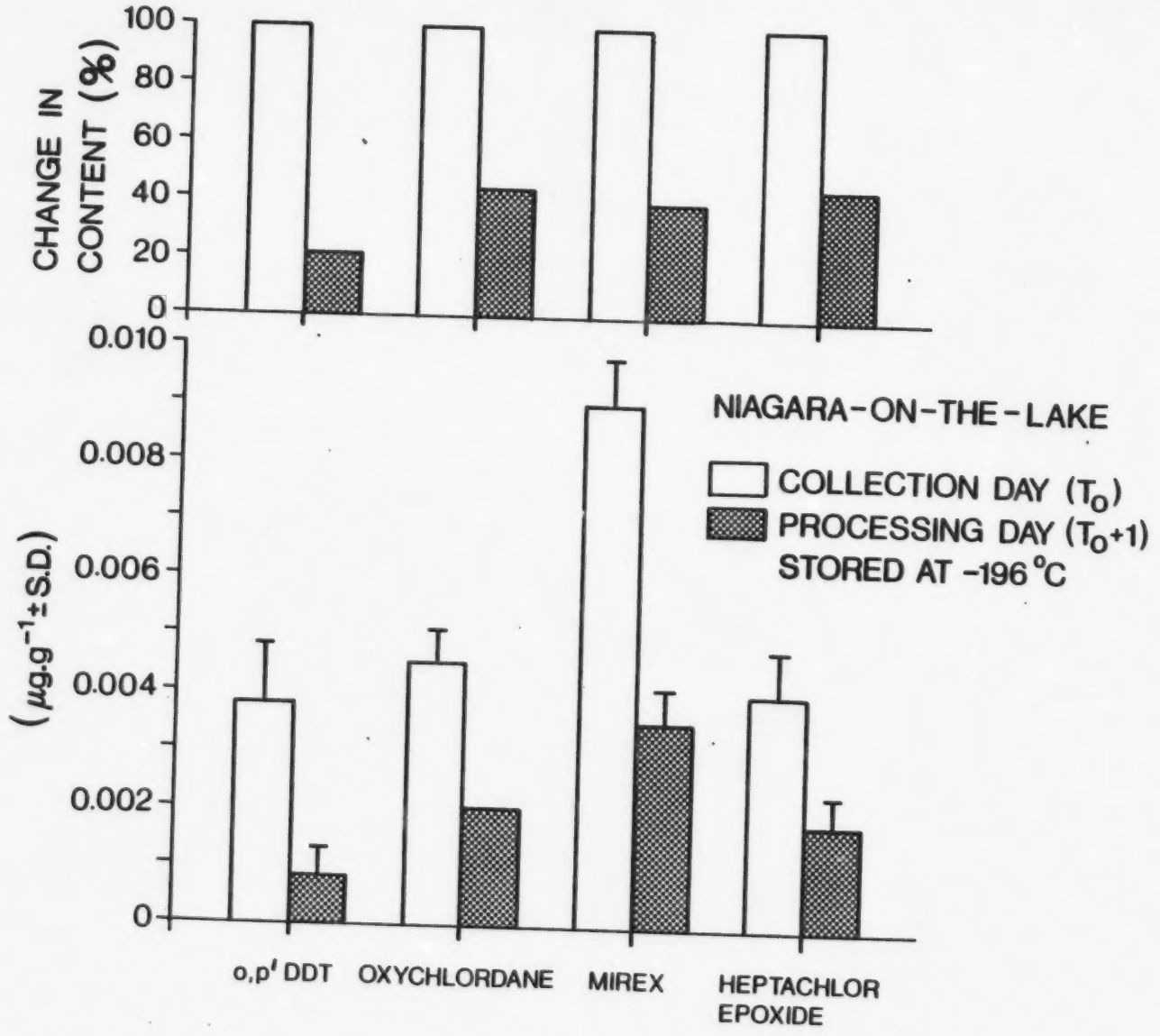

Figure 7. Levels of chlorinated hydrocarbons in Mysis from Niagara-on-the-Lake.

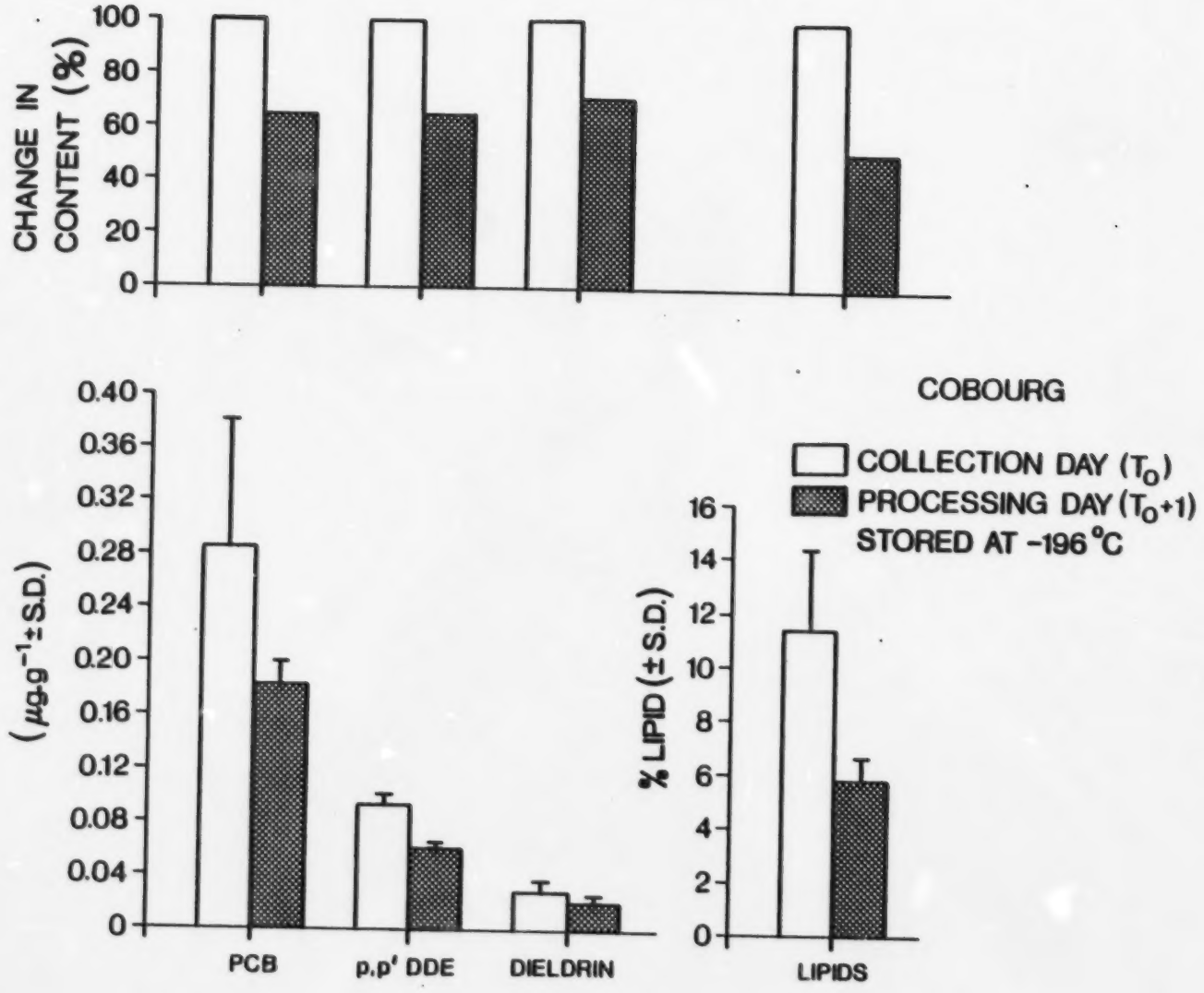

Figure 8. Levels of chlorinated hydrocarbons, PCB and lipid in Mysis from Cobourg.

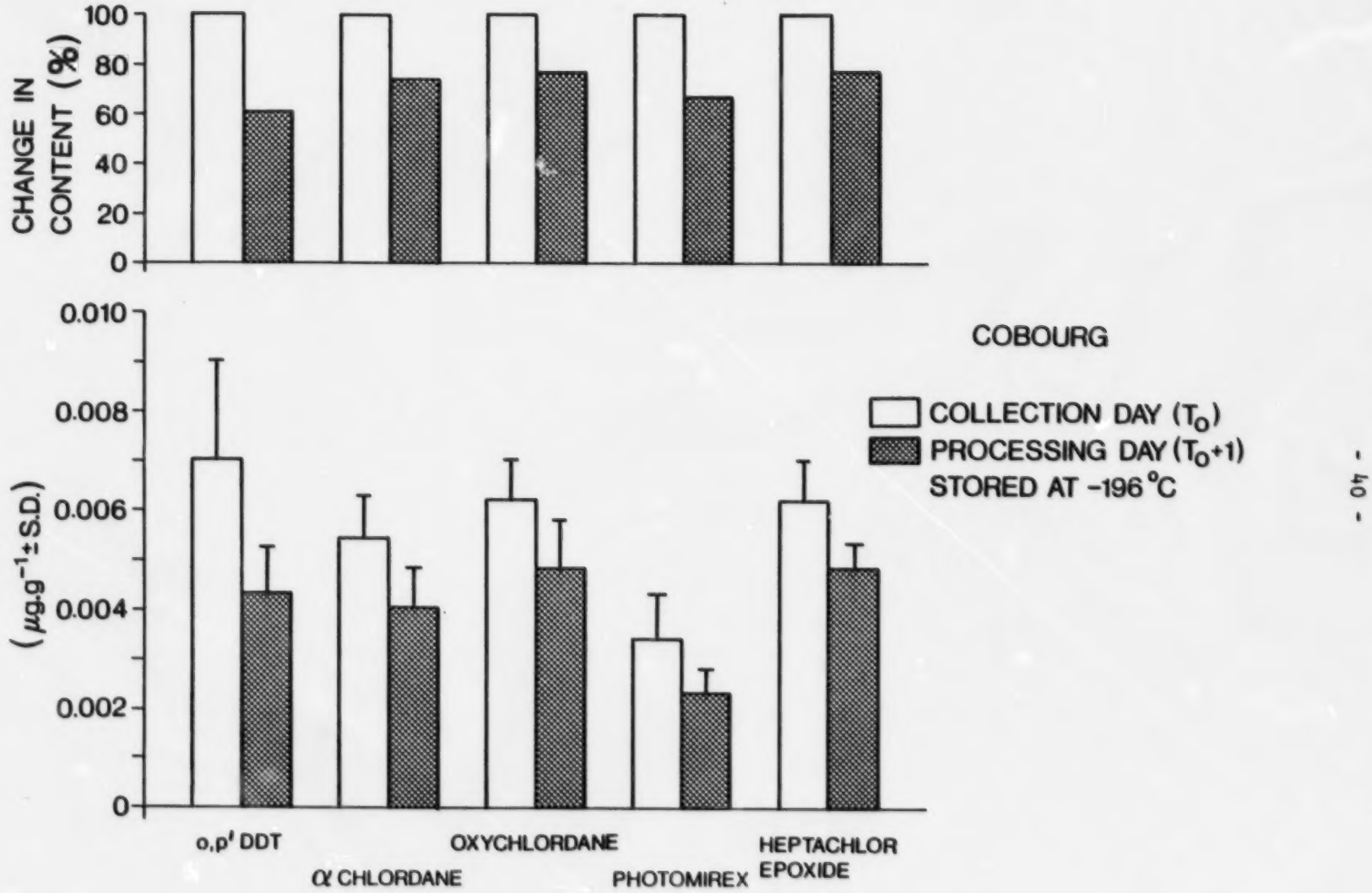

Figure 9. Chlorinated hydrocarbon residue levels in Mysis from Cobourg.

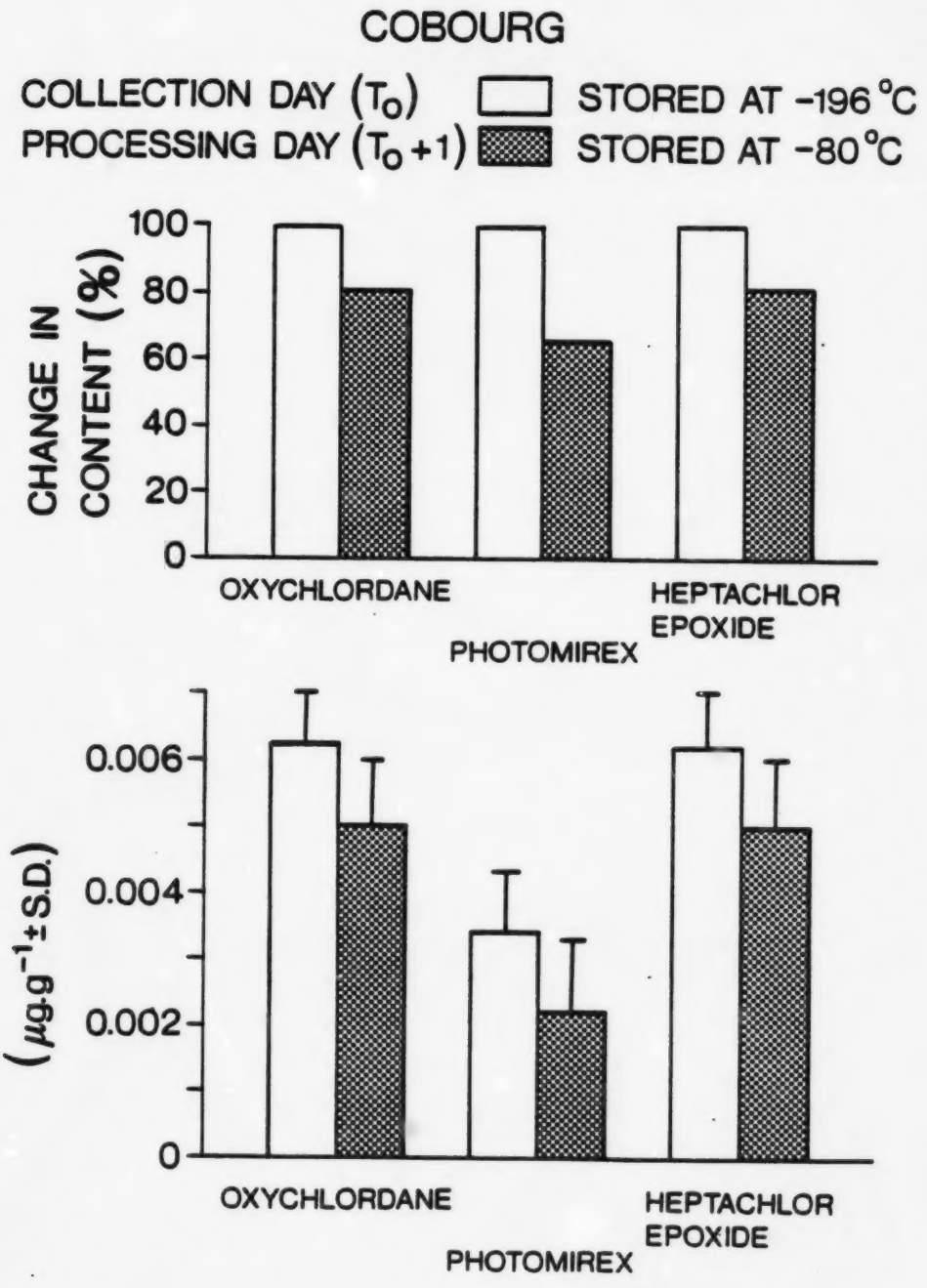

Figure 10. Levels of chlorinated hydrocarbons in Mysis from Cobourg.

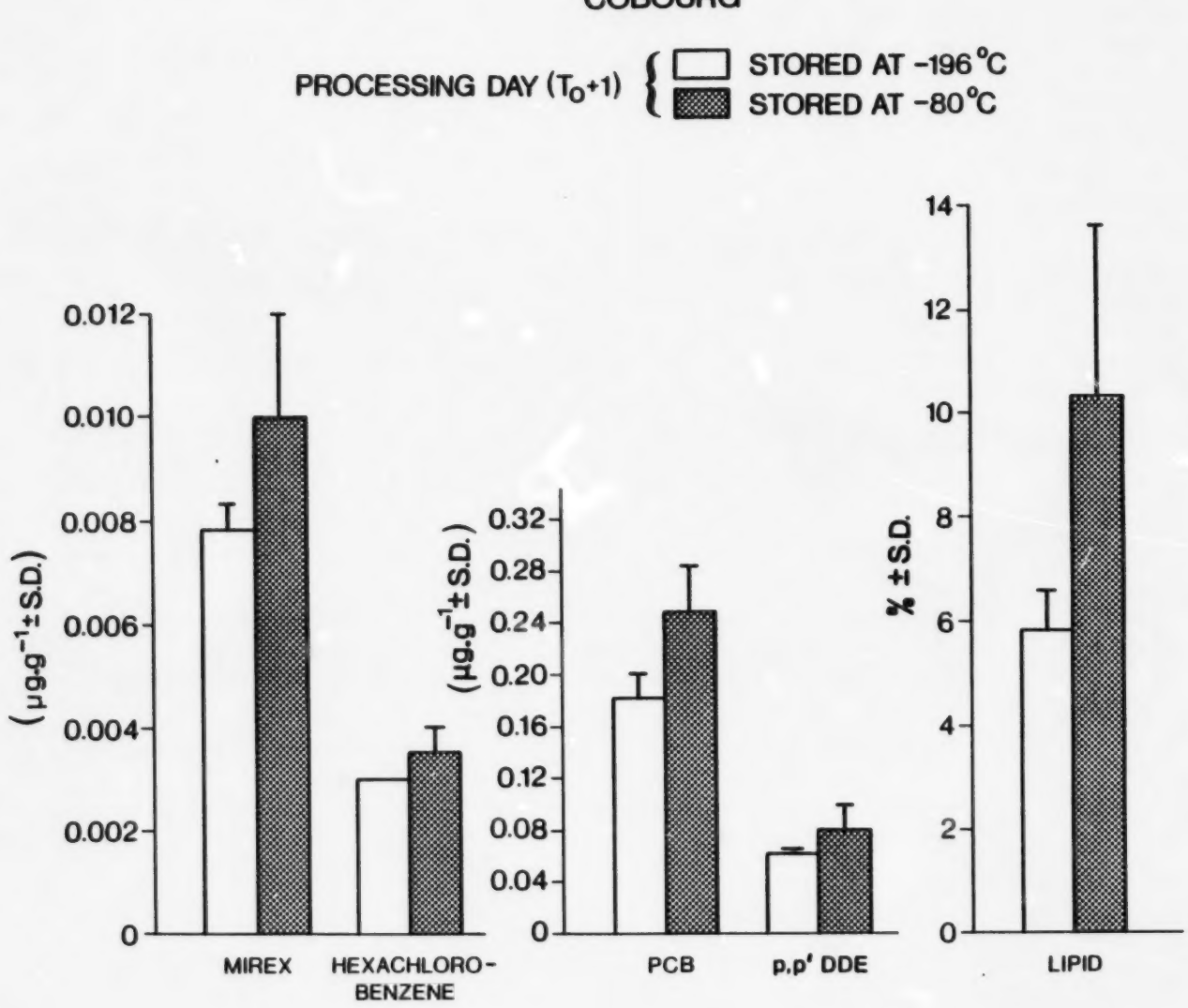

Figure 11. Levels of some chlorinated hydrocarbon residues, PCB and lipid in Mysis from Cobourg.

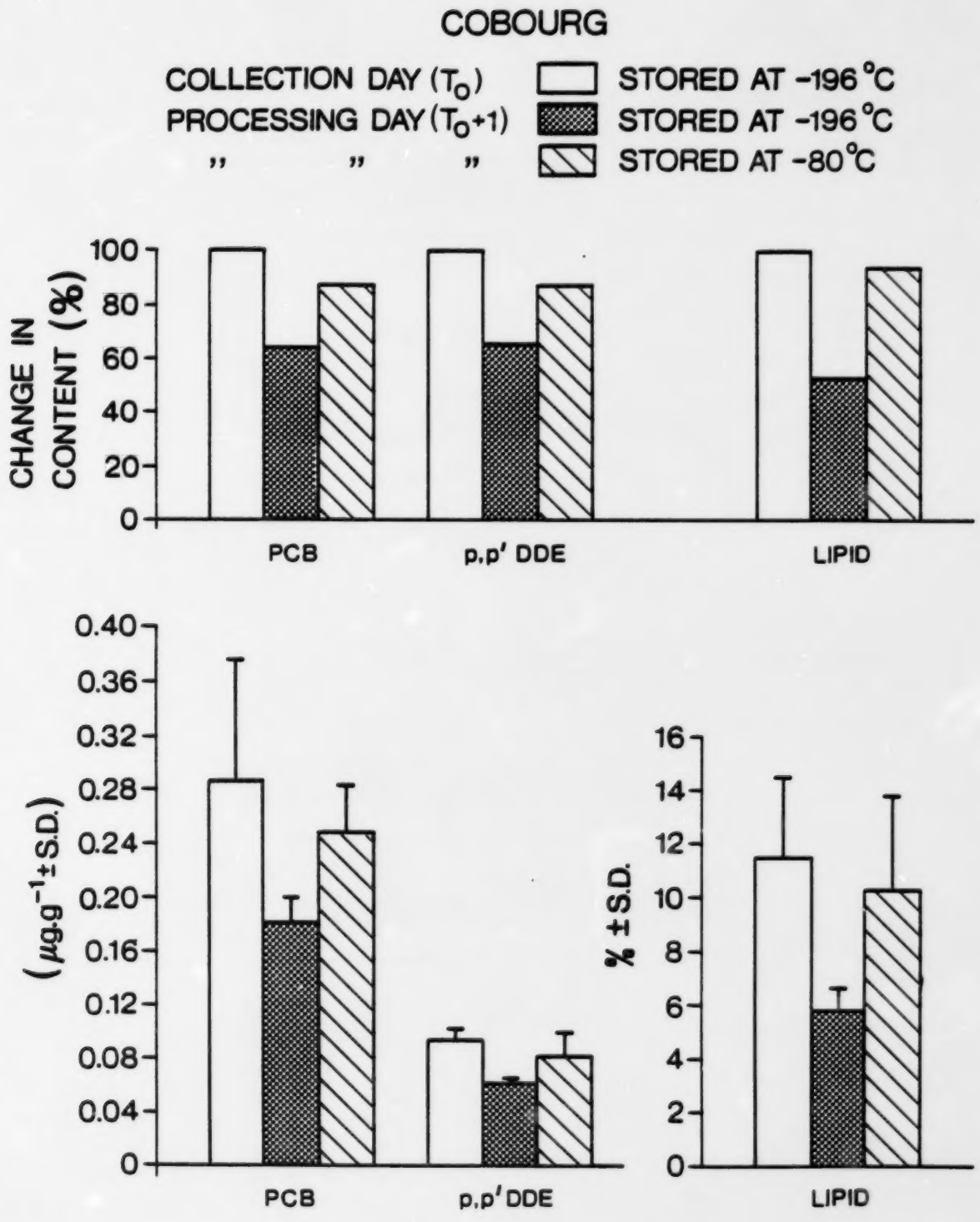

Figure 12. Levels of PCB, p,p'DDE and lipid in Mysis.

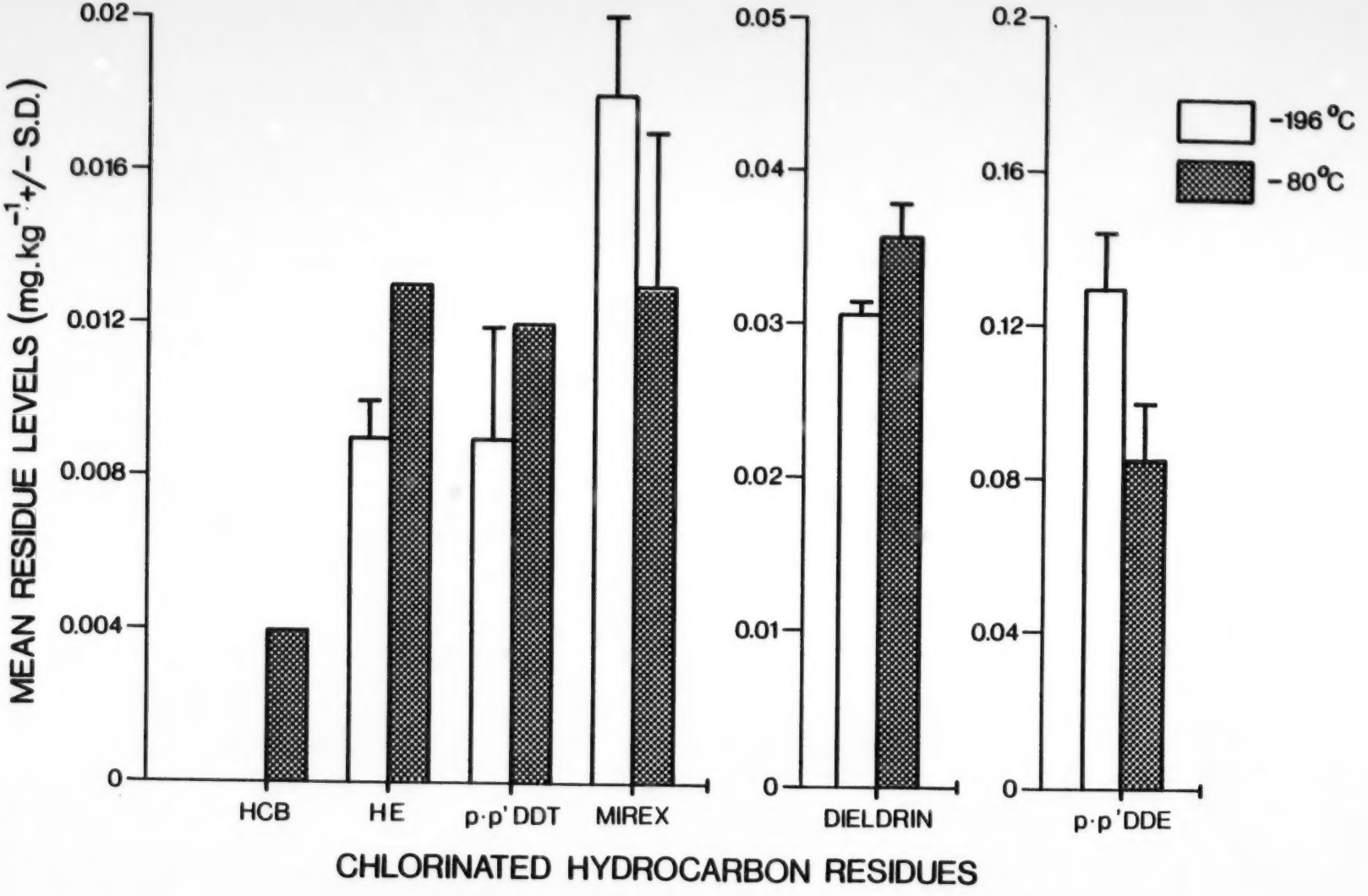

Figure 13. Residue levels in Mysis stored on collection day at $-196^{o}C$ or $-80^{o}C$.

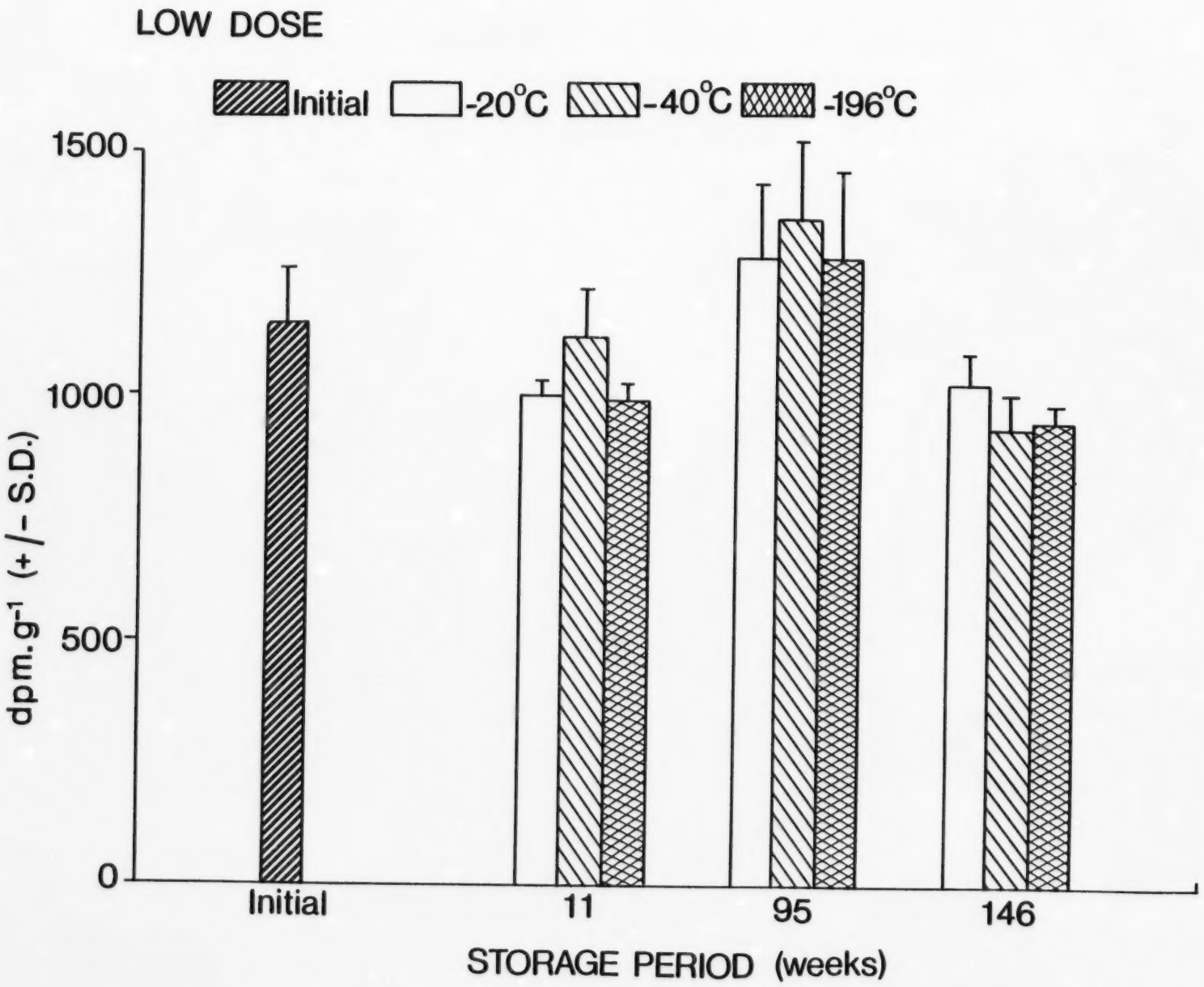

Figure 14. Levels of radio-labelled PCB recovered when extracted with a biological material oxidizer.

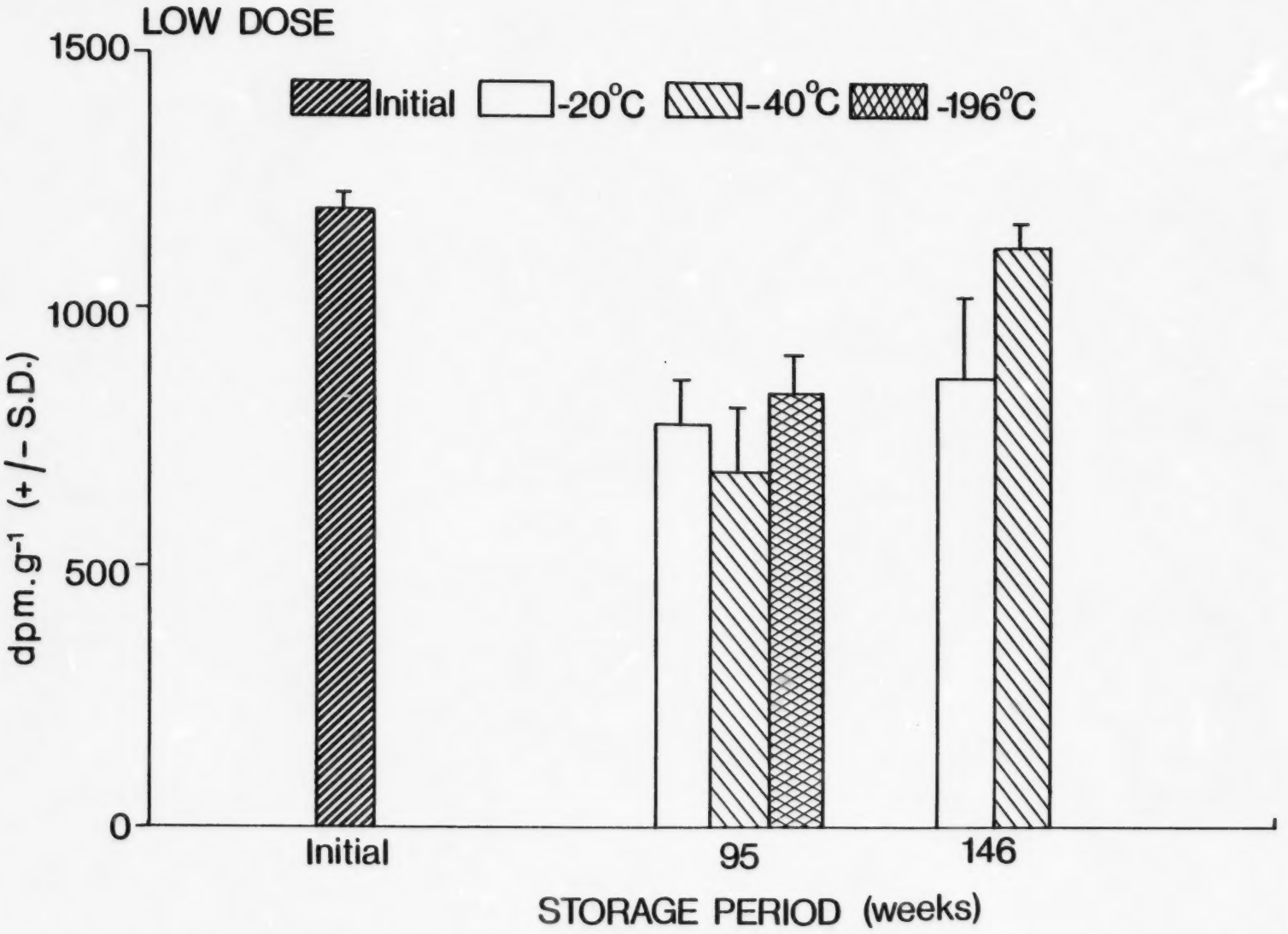

Figure 15. Levels of radio-labelled PCB recovered when extracted with HCl / Methylene Chloride.

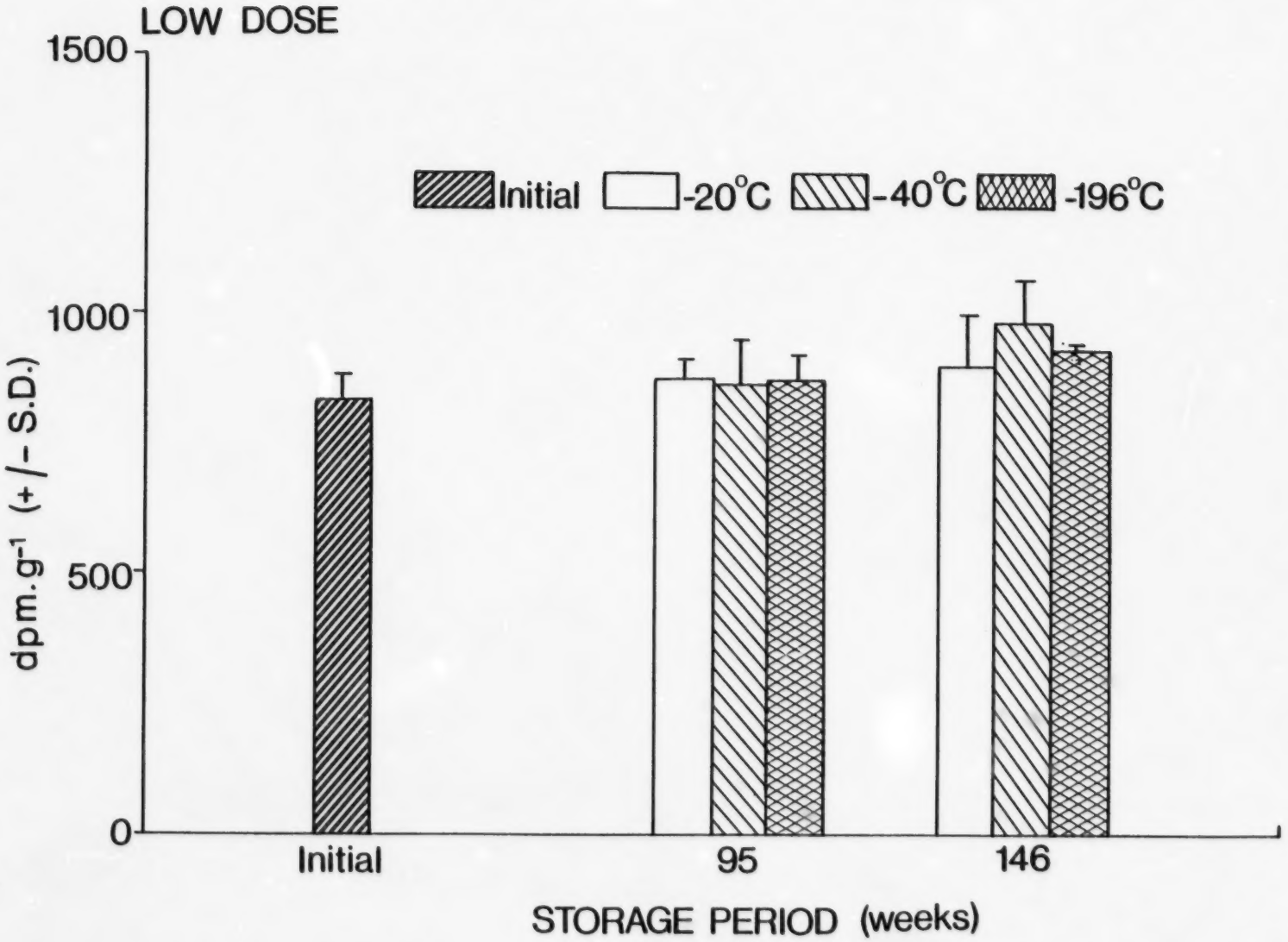

Figure 16. Levels of radio-labelled PCB recovered when extracted with acetonitrile.

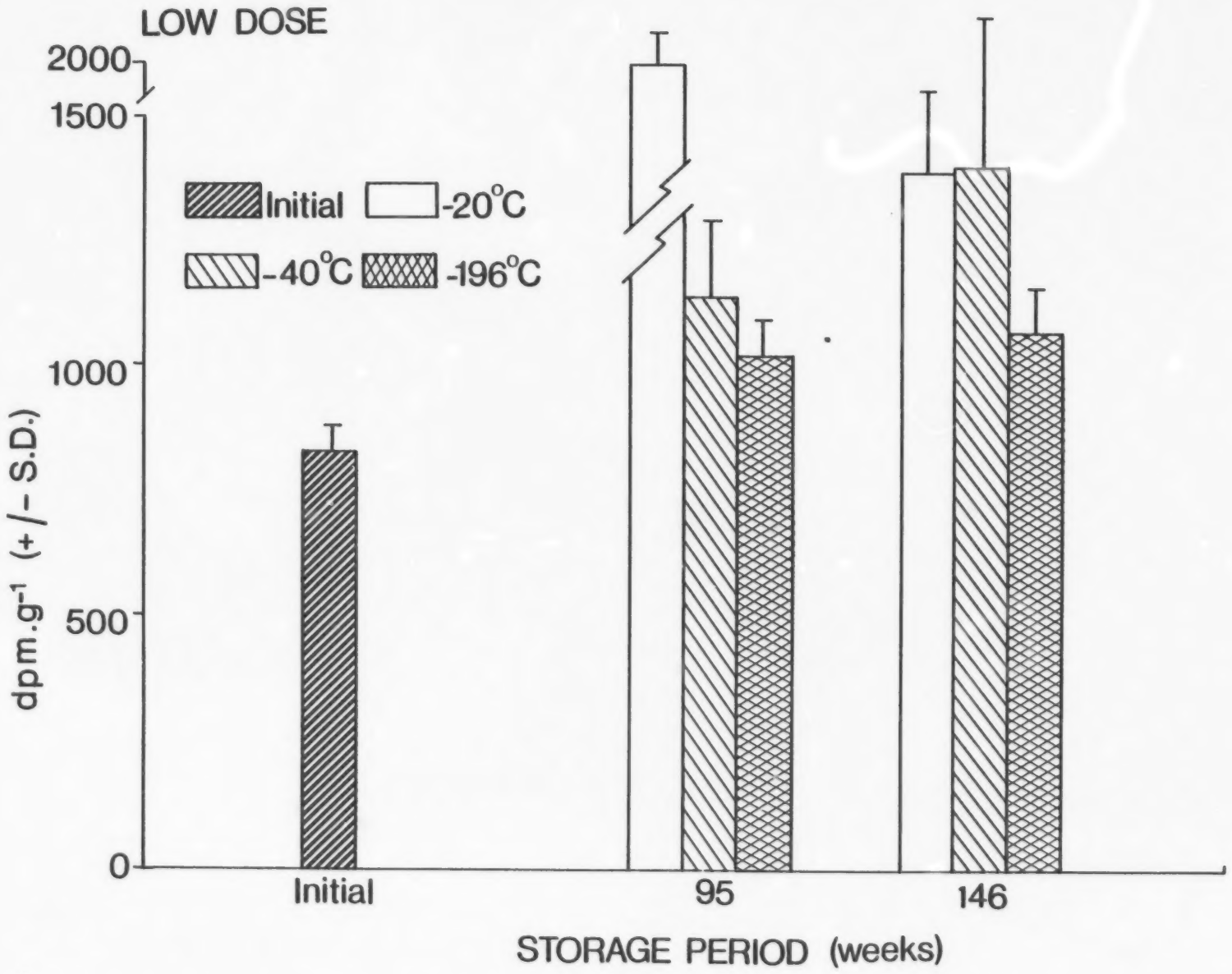

Figure 17. Levels of radio-labelled PCB recovered when extracted with acetone / hexane.

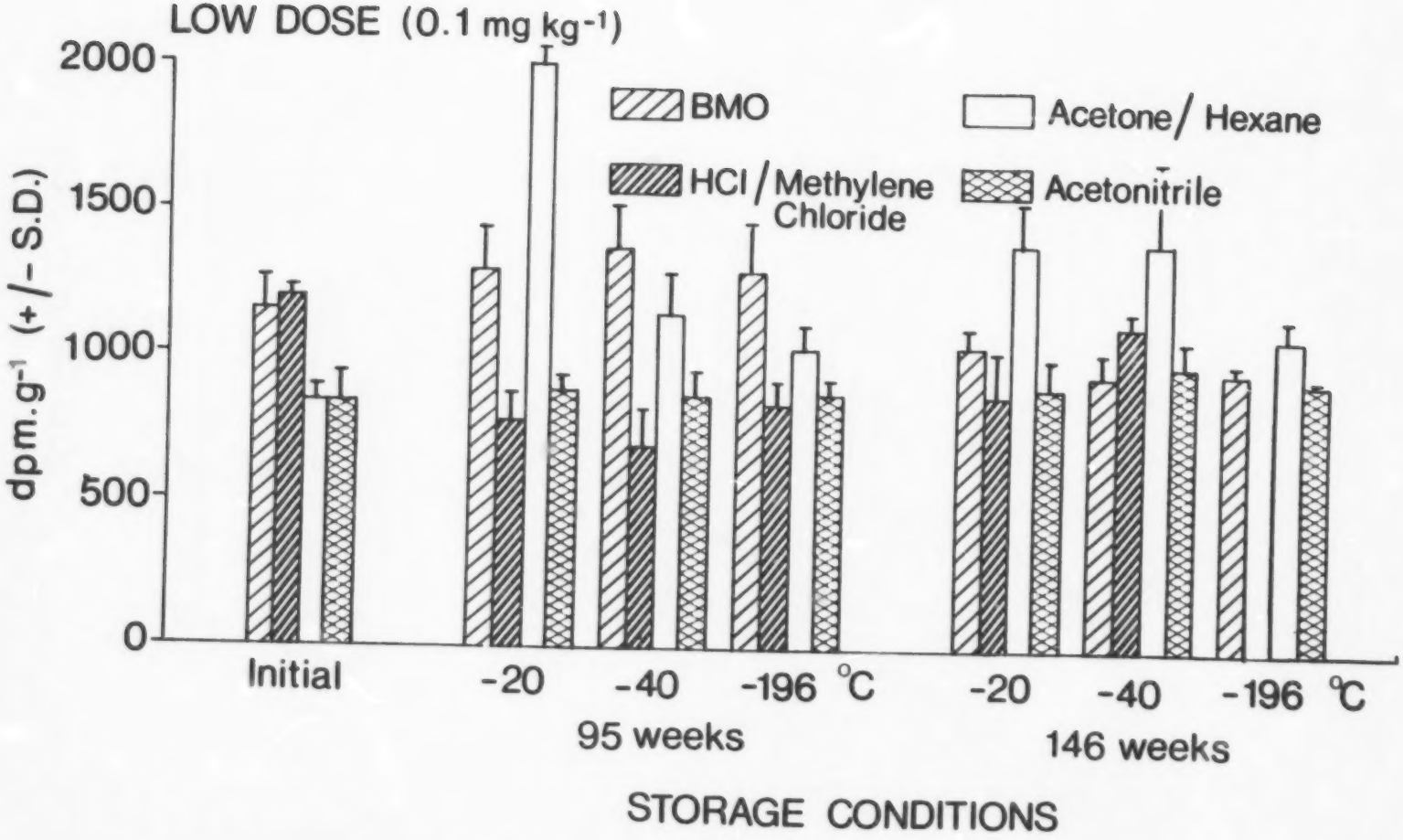

Figure 18. The efficiences of four extraction methods using radio-labelled PCB.

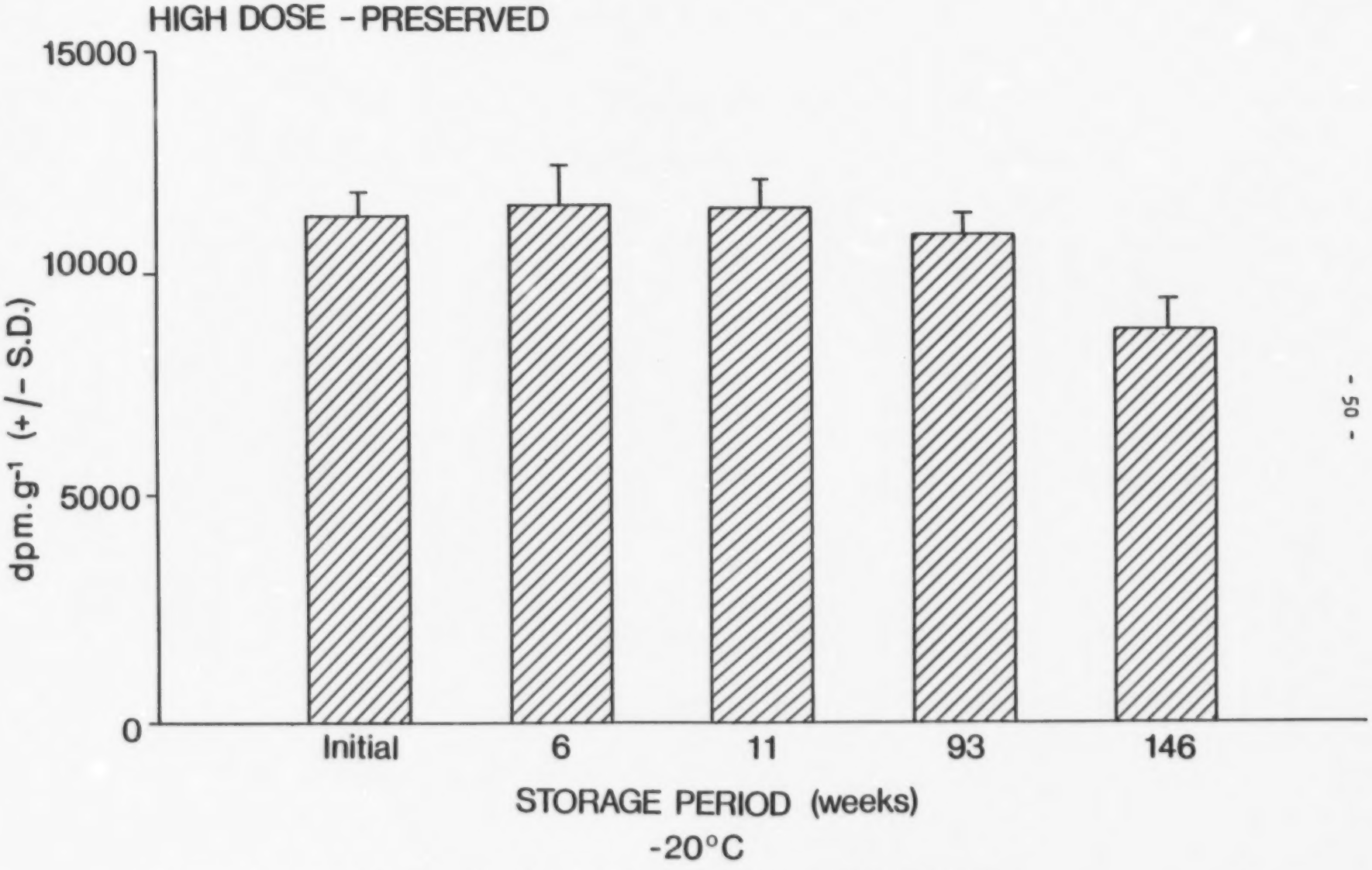

Figure 19. Levels of radio-labelled PCB in preserved fish tissue determined by using a biological material oxidizer.

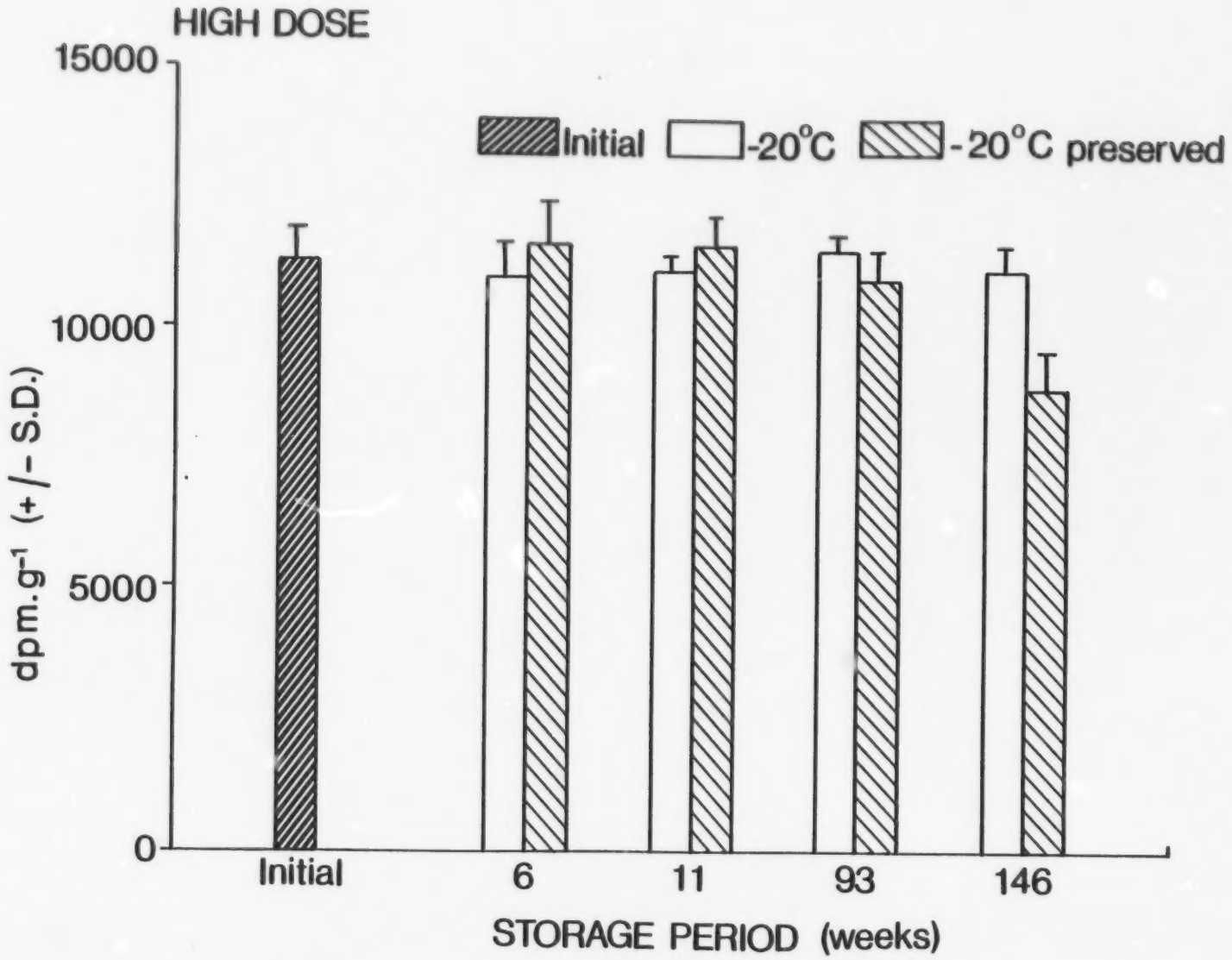

Figure 20. Levels of radio-labelled PCB in fish tissue determined by using a biological material oxidizer.

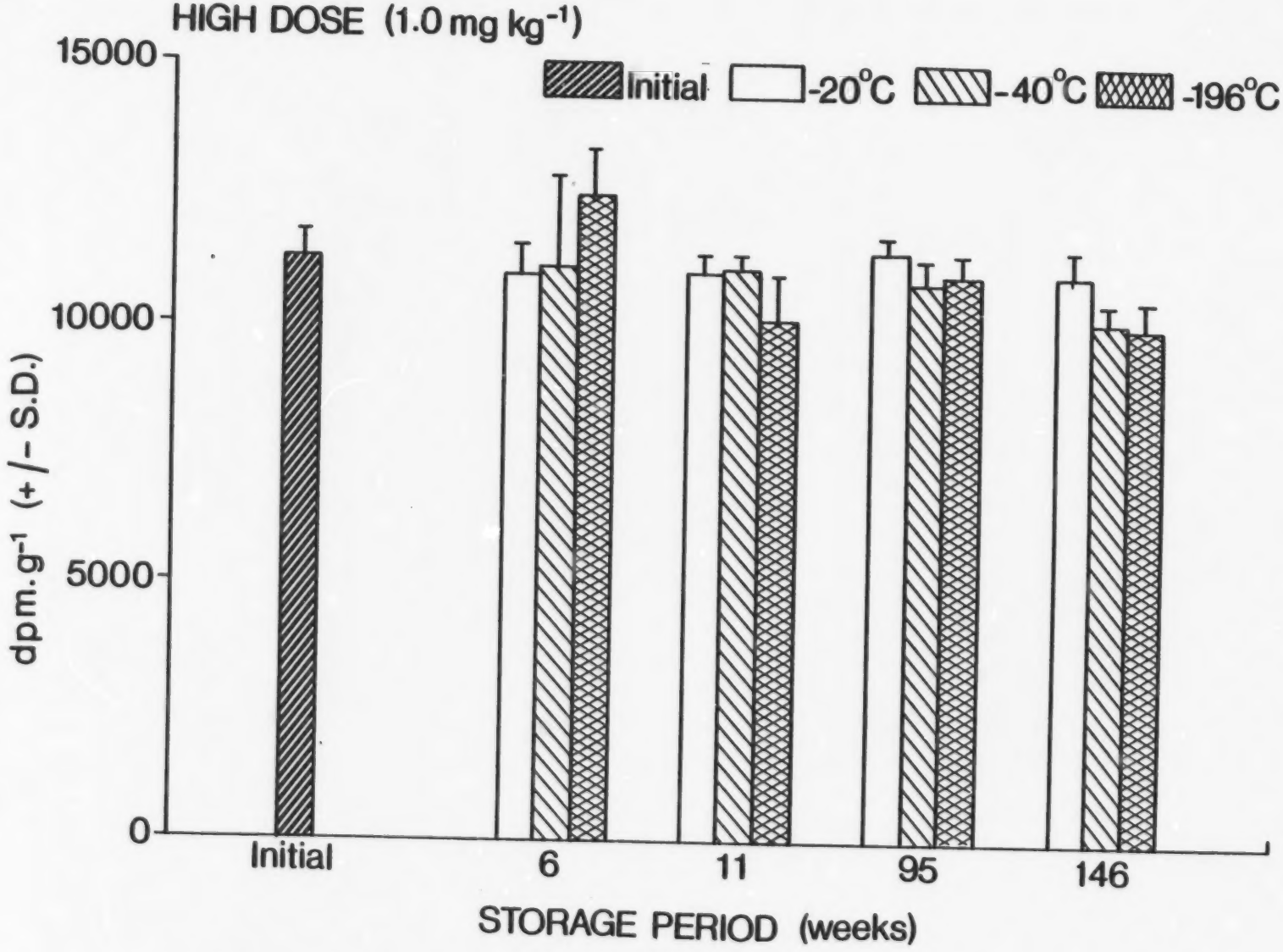

Figure 21. Levels of radio-labelled PCB recovered from fish tissue by using a biological material oxidizer.

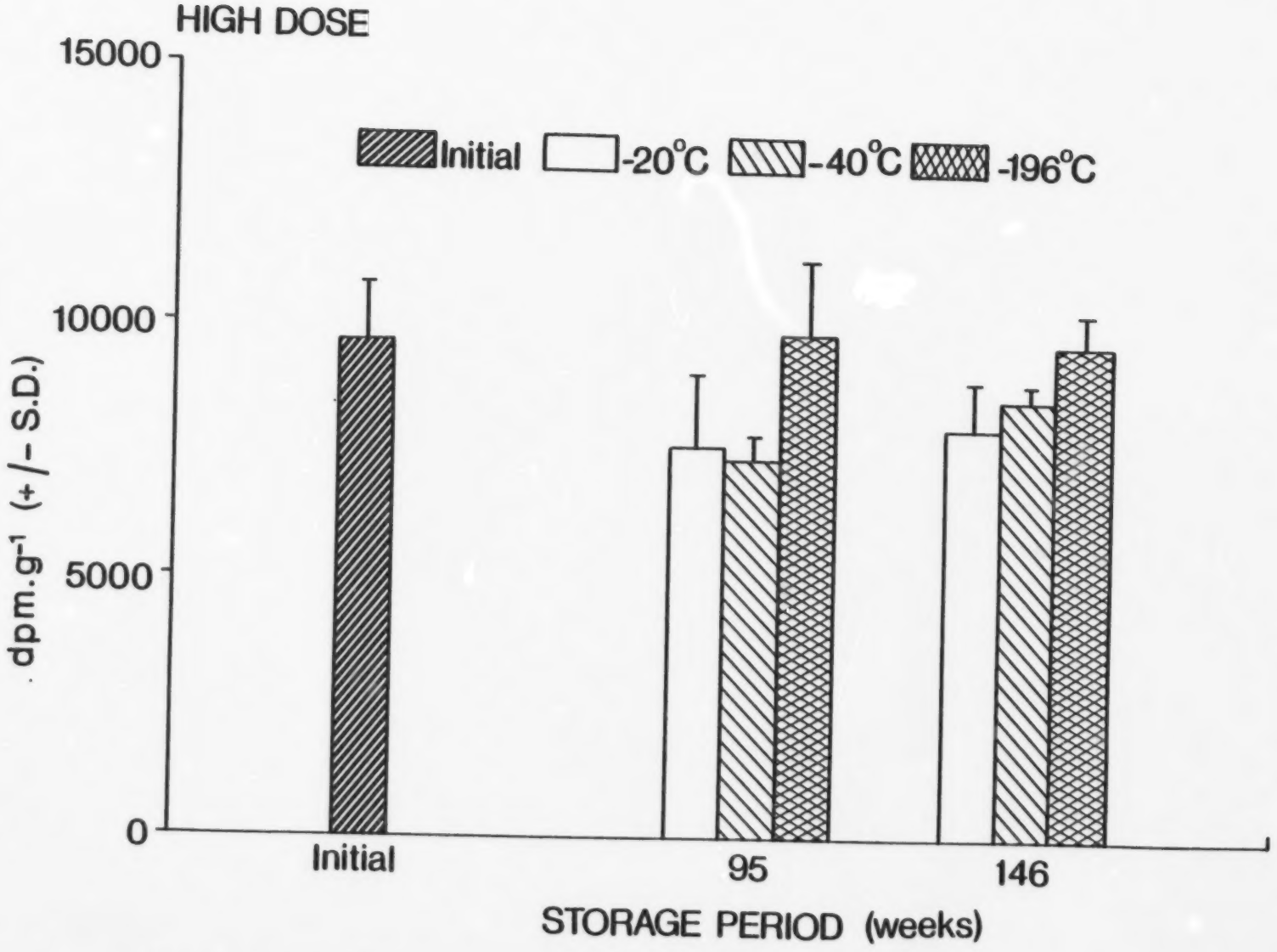

Figure 22. Levels of radio-labelled PCB recovered when extracted with HCl / Methylene Chloride.

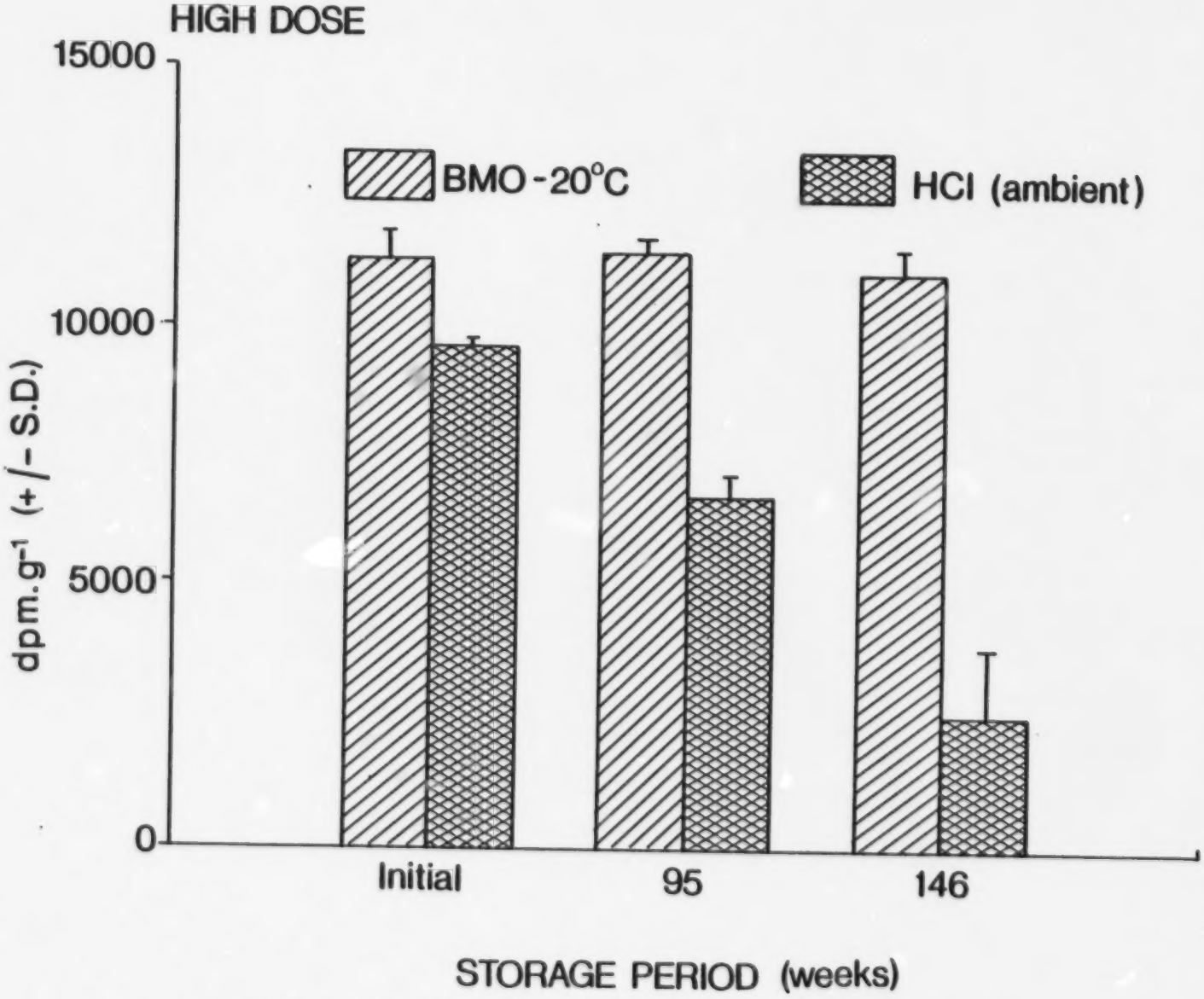

Figure 23. Levels of radio-labelled PCB in fish tissue after acid storage.

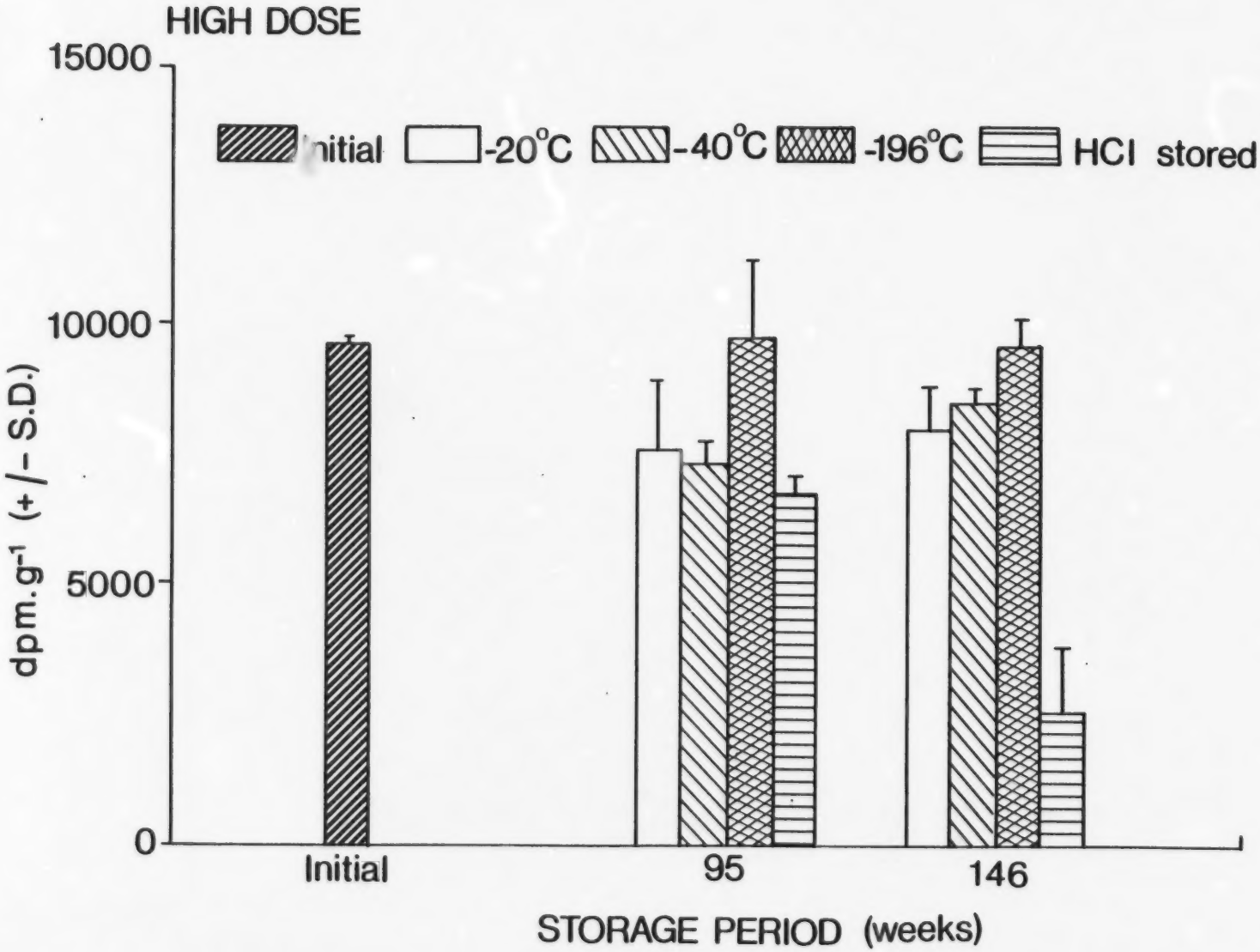

Figure 24. Levels of radio-labelled PCB in samples stored in HCl or frozen, then extracted with HCl.

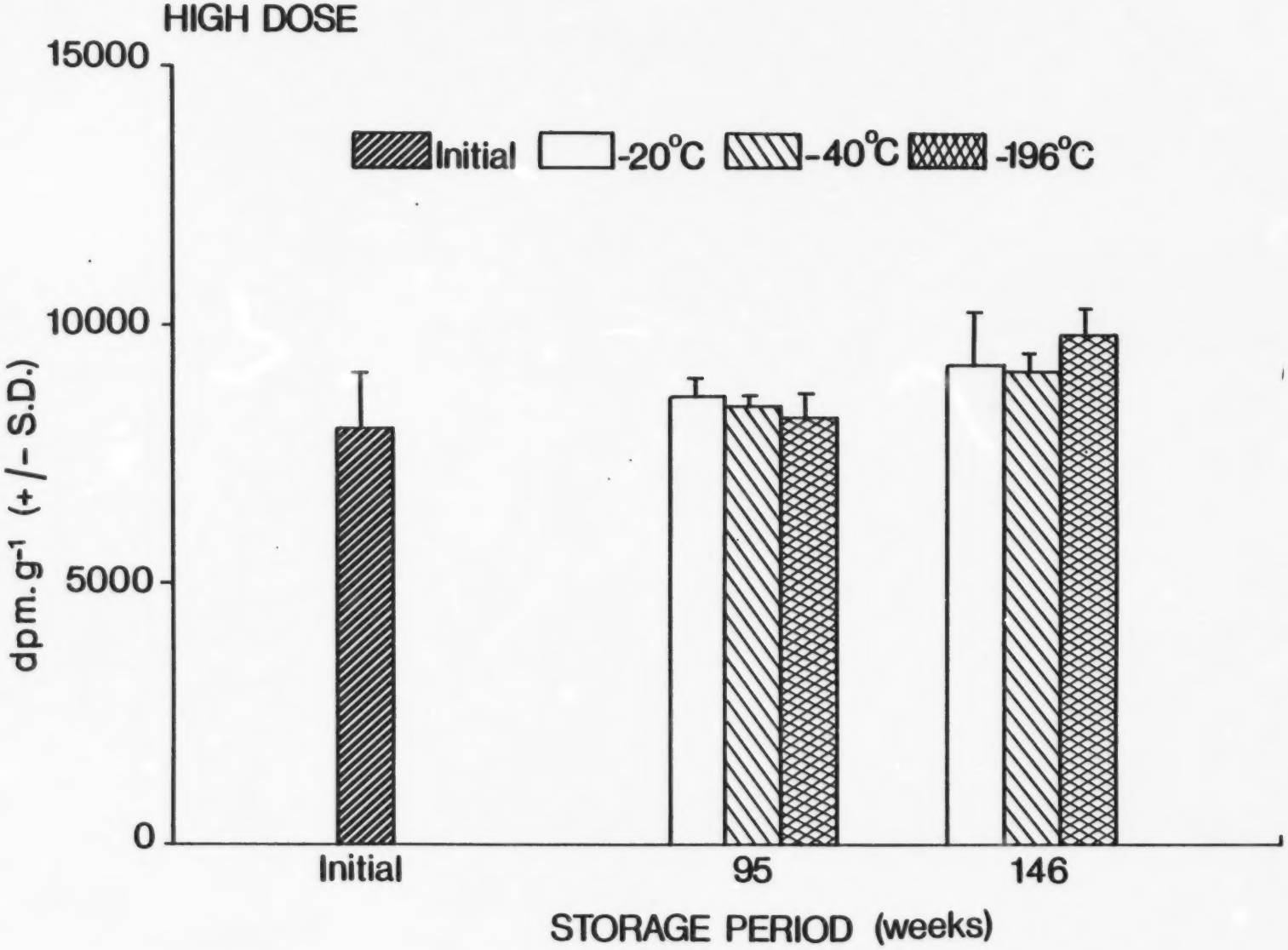

Figure 25. Levels of radio-labelled PCB recovered when extracted with acetone / hexane.

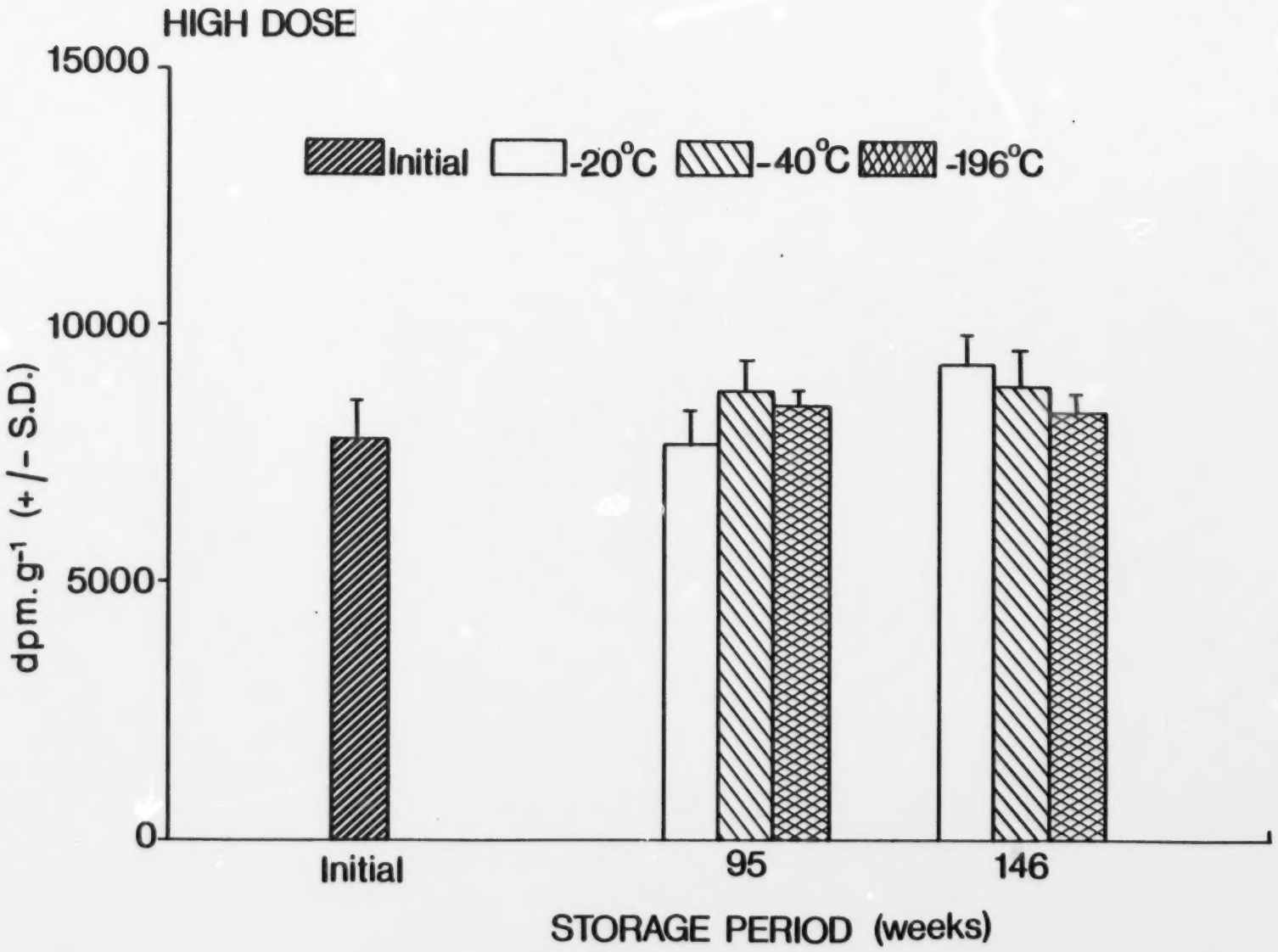

Figure 26. Levels of radio-labelled PCB recovered when extracted with acetonitrile.

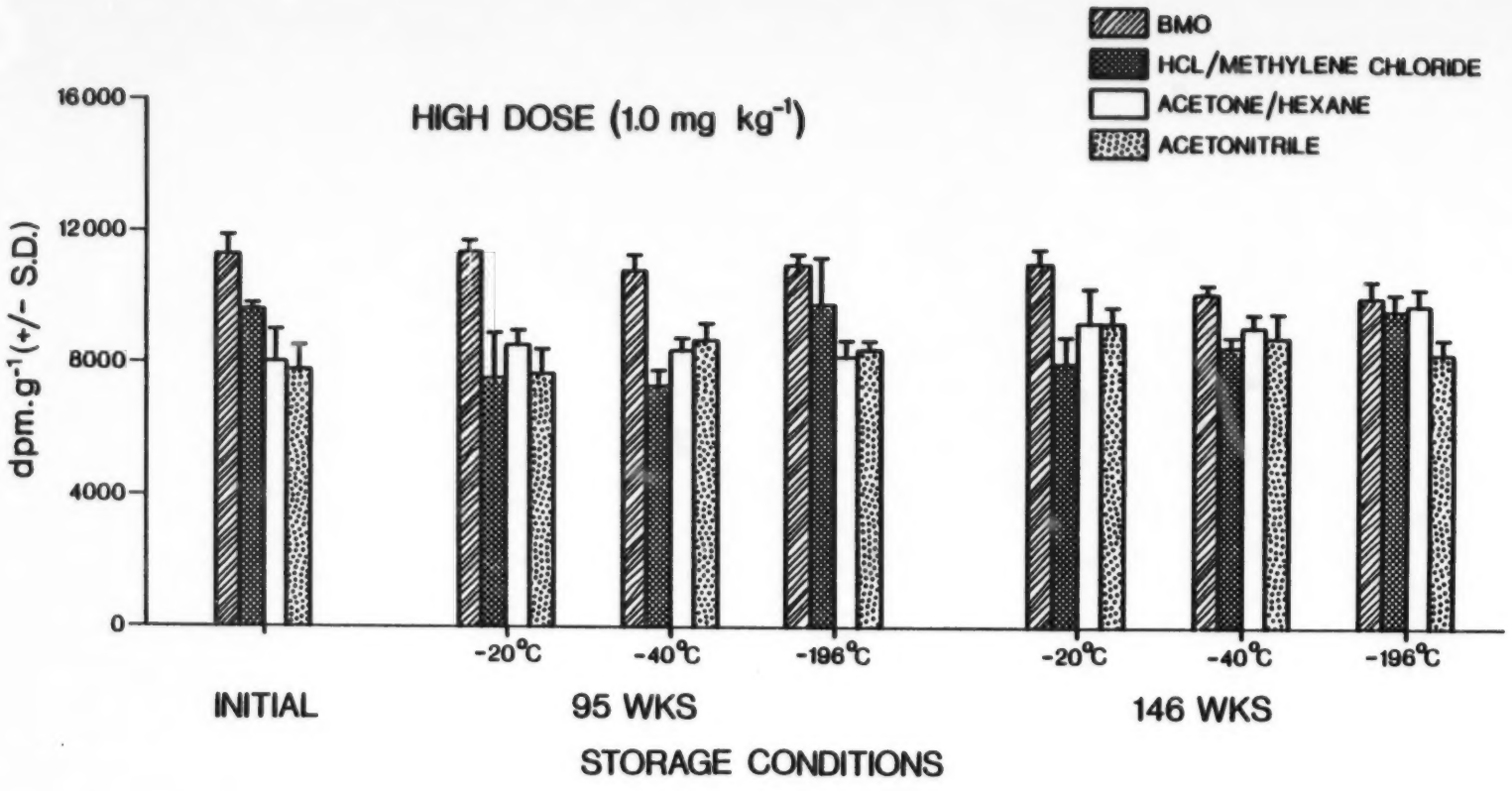

Figure 27. The efficiences of four extraction methods using radio-labelled PCB.